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(54) Title: APPARATUS AND METHOD FOR ANALYZING A FLUID SAMPLE

(57) Abstract: A cartridge for separating a desired analyte from a fluid sample has a sample flow path and a lysing chamber (86) in the sample flow path. The lysing chamber contains at least one filter for capturing cells or viruses from the sample as the sample flows through the lysing chamber. Beads are also disposed in the lysing chamber for rupturing the cells or viruses to release the analyte therefrom. An analyte flow path extends from the lysing chamber and diverges from the sample flow path. The analyte flow path preferably leads to a reaction chamber for chemically reacting and optically detecting the analyte. The cartridge also includes at least one flow controller (e.g., valves) for directing the sample into the waste chamber after the sample flows through the lysing chamber and for directing the analyte separated from the sample into the analyte flow path.

**APPARATUS AND METHOD FOR ANALYZING A FLUID SAMPLE****'FIELD OF THE INVENTION**

5       The present invention relates generally to the field of biochemical analysis, and in particular to an apparatus and method for analyzing a fluid sample to determine the presence or absence of a target analyte in the sample.

**10      BACKGROUND OF THE INVENTION**

The analysis of clinical or environmental fluid samples generally involves a series of chemical, optical, electrical, mechanical, or thermal processing steps on the samples. In recent years, there has been growing interest 15     in developing disposable cartridges for conducting analyses of biological samples for various diagnostic and monitoring purposes. For example, U.S. Patent 5,587,128 to Wilding discloses devices for amplifying a preselected polynucleotide in a sample by conducting a polynucleotide 20     amplification reaction. U.S. Patent 5,922,591 to Anderson et al. describes a miniaturized, integrated nucleic acid diagnostic device and system. The device is generally capable of performing one or more sample acquisition and preparation operations, in combination with one or more 25     sample analysis operations.

Prior fluidic cartridges for processing fluid samples have focused on picoliter, nanoliter, and microliter sample volumes. These small sample volumes are not practical for 30     many realistic diagnostic applications. Of special interest is the detection of target analytes (e.g., nucleic acid) that exist in low concentrations in many samples. For example, in detecting infectious diseases, gram negative bacteria can be present at less than 10 copies per 35     milliliter of blood, cryptosporidium generally appears as only a few copies per gallon of drinking water, concentrated biothreat agents (e.g., anthrax) at less than

100 copies per milliliter of water, and food poisoning agents, such as E. coli and salmonella, may be manifested in less than 10 copies per gram of food.

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#### SUMMARY

The present invention provides an apparatus and method for analyzing a fluid sample to determine the presence or absence of an analyte in the sample. The apparatus includes a cartridge for separating a desired analyte from the sample and for holding the analyte for a chemical reaction and optical detection. The apparatus also includes an instrument for receiving the cartridge for sample processing. The desired analyte is typically intracellular material (e.g., nucleic acid, proteins, carbohydrates, lipids, bacteria, or intracellular parasites). In a preferred use, the analyte is nucleic acid which the cartridge separates from the fluid sample and holds for amplification (e.g., using PCR) and optical detection.

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In a preferred embodiment, the cartridge has a sample port for introducing a sample into the cartridge, and a sample flow path extending from the sample port. The cartridge also has a lysing chamber in the sample flow path. The lysing chamber contains at least one filter for capturing cells or viruses from the sample as the sample flows through the lysing chamber. Beads are also disposed in the lysing chamber for rupturing the cells or viruses to release the analyte therefrom. The cartridge also includes a waste chamber in fluid communication with the lysing chamber via the sample flow path for receiving the remaining sample after the sample flows through the lysing chamber. The cartridge further includes an analyte flow path extending from the lysing chamber. The analyte flow

path diverges from the sample flow path. In the preferred embodiment, the analyte flow path leads to a reaction chamber for chemically reacting and optically detecting the analyte. The cartridge also includes at least one flow controller (e.g., valves) for directing the sample into the waste chamber after the sample flows through the lysing chamber and for directing the analyte separated from the sample into the analyte flow path. The design of the cartridge permits the efficient processing of large sample volumes to enable the accurate detection of low concentration analytes.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is an isometric view of a cartridge for analyzing a fluid sample according to a first embodiment of the invention.

Fig. 2 is a lower isometric view of the cartridge of Fig. 1.

Fig. 3 is an exploded view of the cartridge of Fig. 1.

Fig. 4 is another exploded view of the cartridge of Fig. 1.

Fig. 5 is a partially cut away view of an ultrasonic horn coupled to a wall of a lysing chamber formed in the cartridge of Fig. 1.

Fig. 6 is an exploded view of a filter stack positioned in the lysing chamber of the cartridge of Fig. 1.

Fig. 7 is a top plan view of the cartridge of Fig. 1.

Fig. 8 is a bottom plan view of the cartridge of Fig. 1.

Fig. 9 is a schematic block diagram of the cartridge of Fig. 1.

Fig. 10 is an isometric view of an instrument into which the cartridge of Fig. 1 is placed for processing.

Fig. 11 is an isometric view of the cartridge of Fig. 1 in the instrument of Fig. 10.

Fig. 12 is a partially cut-away view of the cartridge of Fig. 1 in the instrument of Fig. 10.

Fig. 13 is a schematic, plan view of optical sensors positioned to detect liquid levels in the cartridge of Fig. 1.

Fig. 14 is a partially cut away, schematic, side view of a slotted optical sensor positioned to detect the liquid level in a sensor chamber of the cartridge of Fig. 1.

Fig. 15A is a cross-sectional view of a portion of the body of the cartridge of Fig. 1 illustrating two different types of valves in the cartridge.

Fig. 15B is a cross-sectional view of the valves of Fig. 15A in a closed position.

Fig. 16A is another cross-sectional view of one of the valves of Fig. 15A in an open position.

Fig. 16B is a cross-sectional view of the valve of Fig. 16A in a closed position.

Figs. 17-19 illustrate a valve actuation system for opening and closing the valves of Fig. 15A.

Fig. 20 is a cross sectional view of alternative valve actuators for opening and closing the valves in the cartridge of Fig. 1. Fig. 20 also shows a pressure delivery nozzle sealed to a pressure port formed in the cartridge of Fig. 1.

Fig. 21 is a partially exploded, isometric view of a reaction vessel of the cartridge of Fig. 1.

Fig. 22 is a front view of the vessel of Fig. 21.

Fig. 23 is a side view of the vessel of Fig. 21 inserted between two heater plates.

Fig. 24 is a front view of one of the heater plates of Fig. 23.

Fig. 25 is a front view of an alternative reaction vessel according to the present invention.

Fig. 26 is a front view of another reaction vessel according to the present invention.

Fig. 27 is another front view of the vessel of Fig. 21.

Fig. 28 is a front view of the vessel of Fig. 21 inserted into a heat-exchanging module of the instrument of Fig. 10.

Fig. 29 is an exploded view of a support structure for holding the plates of Fig. 23.

Figs. 30-31 are assembled views of the support structure of Fig. 29.

5 Fig. 32 is an isometric view showing the exterior of one the optics assemblies in the heat-exchanging module of Fig. 28.

Fig. 33 is an isometric view of the plates of Fig. 23 in contact with the optics assembly of Fig. 32.

10 Fig. 34 is a partially cut away, isometric view of the reaction vessel of Fig. 21 inserted between the plates of Fig. 23. Only the lower portion of the vessel is included in the figure.

Fig. 35 is a schematic block diagram of the electronics of  
15 the heat-exchanging module of Fig. 28.

Fig. 36 is an isometric view of an apparatus for disrupting cells or viruses according to another embodiment of the invention..

Fig. 37 is a cross sectional view of the apparatus of Fig.  
20 36.

Fig. 38 is an exploded view of a container used in the apparatus of Fig. 36.

Fig. 39 is a cross sectional view of the container of Fig.  
38.

25 Fig. 40 is a schematic block diagram of a fluidic system incorporating the apparatus of Fig. 36.

Fig. 41 is a cross sectional view of another container for use in the apparatus of Fig. 36. An ultrasonic horn is in contact with a wall of the container  
30 that curves outwardly towards the horn.

Fig. 42 is a cross-sectional view of the wall of Fig. 41.

Figs. 43A-43B are isometric views of opposite sides of another wall suitable for use in a container for holding cells or viruses to be disrupted.

Fig. 44 is a partially cut-away, isometric view of a container incorporating the wall of Figs. 43A-43B.

Fig. 45 is a bottom plan view of the container of Fig. 44.

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#### DETAILED DESCRIPTION

The present invention provides an apparatus and method for analyzing a fluid sample. In a first embodiment, the invention provides a cartridge for separating a desired analyte from a fluid sample and for holding the analyte for 10 a chemical reaction. The fluid sample may be a solution or suspension. In a particular use, the sample may be a bodily fluid (e.g., blood, urine, saliva, sputum, seminal fluid, spinal fluid, mucus, or other bodily fluids). Alternatively, the sample may be a solid made soluble or 15 suspended in a liquid or the sample may be an environmental sample such as ground or waste water, soil extracts, pesticide residues, or airborne spores placed in a fluid. Further, the sample may be mixed with one or more chemicals, reagents, diluents, or buffers. The sample may 20 be pretreated, for example, mixed with chemicals, centrifuged, pelleted, etc., or the sample may be in a raw form.

The desired analyte is typically intracellular material 25 (e.g., nucleic acid, proteins, carbohydrates, lipids, bacteria, or intracellular parasites). In a preferred use, the analyte is nucleic acid which the cartridge separates from the fluid sample and holds for amplification (e.g., using PCR) and optical detection. As used herein, the term 30 "nucleic acid" refers to any synthetic or naturally occurring nucleic acid, such as DNA or RNA, in any possible configuration, i.e., in the form of double-stranded nucleic acid, single-stranded nucleic acid, or any combination thereof.

Fig. 1 shows an isometric view of a cartridge 20 according to the preferred embodiment. The cartridge 20 is designed to separate nucleic acid from a fluid sample and to hold 5 the nucleic acid for amplification and detection. The cartridge 20 has a body comprising a top piece 22, a middle piece 24, and a bottom piece 26. An inlet port for introducing a fluid sample into the cartridge is formed in the top piece 22 and sealed by a cap 30. Six pressure 10 ports 32 are also formed in the top piece 22. The pressure ports 32 are for receiving nozzles from pressure sources, e.g., pumps or vacuums. The cartridge also includes alignment legs 28 extending from the bottom piece 26 for positioning the cartridge 20 in an instrument (described 15 below with reference to Fig. 10). Indentations or depressions 38A, 38B, and 38C are formed in the top and middle pieces 22, 24. The indentations are for receiving optical sensors that detect fluid flow in the cartridge 20. The cartridge 20 further includes vents 34, 36. Each 20 pressure port and vent preferably includes a hydrophobic membrane that allows the passage of gas but not liquid into or out of the vents and pressure ports. Modified acrylic copolymer membranes are commercially available from, e.g., Gelman Sciences (Ann Arbor, MI) and particle-track etched 25 polycarbonate membranes are available from Poretics, Inc. (Livermore, CA).

Fig. 2 is an isometric view showing the underside of the cartridge 20. Nine holes 60 are formed in the bottom piece 30 26 for receiving valve actuators that open and close valves in the cartridge 20. A hole 62 is also formed in the bottom piece 26 for receiving a transducer (described in detail below with reference to Fig. 5). The cartridge 20 also includes a reaction vessel 40 extending outwardly from 35 the body of the cartridge. The vessel 40 has a reaction chamber 42 for holding a reaction mixture (e.g., nucleic acid mixed with amplification reagents and fluorescent probes) for chemical reaction and optical detection. One

of the flow paths in the cartridge carries the reaction mixture to the chamber 42 for chemical reaction and optical detection. The vessel 40 extends outwardly from the body of the cartridge 20 so that the vessel 40 may be inserted

5 between a pair of opposing thermal plates (for heating and cooling the chamber 42) without the need for decoupling the vessel 40 from the rest of the cartridge 20. This greatly reduces the risk of contamination and/or spilling. The vessel 40 may be integrally formed with the body of the

10 cartridge (e.g., integrally molded with middle piece 24). It is presently preferred, however, to produce the vessel 40 as a separate element that is coupled to the body during manufacture of the cartridge.

15 Figs. 3-4 show exploded views of the cartridge. As shown in Fig. 3, the middle piece 24 has multiple chambers formed therein. In particular, the middle piece 24 includes a sample chamber 65 for holding a fluid sample introduced through the inlet port 64, a wash chamber 66 for holding a

20 wash solution, a reagent chamber 67 for holding a lysing reagent, a waste chamber 68 for receiving used sample and wash solution, a neutralizer chamber 70 for holding a neutralizer, and a master mix chamber 71 for holding a master mix (e.g., amplification reagents and fluorescent

25 probes) and for mixing the reagents and probes with analyte separated from the fluid sample. The sample chamber 65 optionally includes a side compartment 155 having slightly lower walls than the sample chamber 65. The side compartment 155 is for visually indicating to a user when

30 sufficient sample has been added to the sample chamber 65, i.e., when the liquid level in the chamber 65 is high enough to spill over into the compartment 155.

The top piece 22 includes the vents 34, 36 and the six

35 pressure ports 32, as previously described. An elastomeric membrane or gasket 61 is positioned and squeezed between the pieces 22, 24 to seal the various channels and chambers formed in the pieces. The middle piece 24 preferably

includes multiple sealing lips to ensure that the gasket 61 forms an adequate seal. In particular, the middle piece 24 preferably includes sealing lips 73 surrounding each of the chambers 65, 66, 67, 68, 70, and 71. The middle piece 24

5 also includes support walls 75 around the perimeter, and intermediate sealing lips 76. The sealing lips 73, 76 and support walls 75 locally compress the gasket 61 and achieve a seal.

10 As shown in Fig. 4, the middle piece 24 has formed in its underside various channels, one of which leads to a lysing chamber 86. The chamber 86 is aligned with the hole 62 in the bottom piece 26 so that a transducer (e.g., an ultrasonic horn) may be inserted through the hole 62 to

15 generate pressure waves in the lysing chamber 86. The middle piece 24 also has nine valve seats 84 formed in its bottom surface. The valve seats 84 are aligned with the nine holes 60 in the bottom piece 26 so that valve actuators may be inserted through the holes 60 into the

20 valve seats 84.

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An elastomeric membrane or gasket 61 is positioned and squeezed between the pieces 24, 26 to seal the various channels, valve seats, and chamber formed in the middle piece 24. The middle piece 24 preferably includes multiple sealing lips to ensure that the gasket 63 forms an adequate seal. In particular, the middle piece 24 preferably includes sealing lips 73 surrounding the lysing chamber 86, valve seats 84, and various channels. The middle piece 24

25 also includes support walls 75 around its perimeter, and intermediate sealing lips 76. The sealing lips 73, 76 and support walls 75 locally compress the gasket 63 and achieve a seal. In addition to sealing various channels and chambers, the gasket 63 also functions as a valve stem by

30 compressing, when actuated through one of the holes 60, into a corresponding valve seat 84, thus shutting one of the flow channels in the middle piece 24. This valve

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action is discussed in greater detail below with reference to Figs. 15-16.

The gasket 63 also forms the bottom wall of the lysing chamber 86 against which a transducer is placed to effect disruption of cells or viruses in the chamber 86. Each of the gaskets 61, 63 is preferably composed of an elastomer. Suitable gasket materials are silicone rubber, neoprene, EPDM, or any other compliant material. Each of the gaskets 61, 63 preferably has a thickness in the range of 0.005 to 0.125 inches (0.125 to 3.175 mm), and more preferably in the range of 0.01 to 0.06 inches (0.25 to 1.5 mm), with a presently preferred thickness of .031 inches (0.79 mm). The thickness is selected to ensure that the gasket is sufficiently compliant to seal the channels and chambers, to compress into the valve seats 84 when forced, and to expand under pressure to contact the transducer.

As shown in Fig. 3, the middle piece 24 includes a slot 79 through which the reaction vessel 40 is inserted during assembly of the cartridge. The vessel 40 has two fluid ports 41, 43 for adding and removing fluid from the vessel. When the top piece 22 is sealed to the middle piece 24 via the gasket 61, the ports 41, 43 are placed into fluidic communication with channels 80, 81, respectively, that are formed in the top piece 22 (see Fig. 4). The gasket 61 seals the respective fluidic interfaces between the ports 41, 43 and the channels 80, 81. The top, middle, and bottom pieces 22, 24, 26 are preferably injection molded parts made of a polymeric material such as polypropylene, polycarbonate, or acrylic. Although molding is preferred for mass production, it also possible to machine the top, middle, and bottom pieces 22, 24, 26. The pieces 22, 24, 26 may be held together by screws or fasteners. Alternatively, ultrasonic bonding, solvent bonding, or snap fit designs could be used to assemble the cartridge.

Fig. 4 also shows a filter ring 88. The filter ring 88 compresses and holds a stack of filters in the lysing chamber 86. Fig. 6 shows an exploded view of a filter stack 87. The purpose of the filter stack 87 is to capture cells or viruses from a fluid sample as the sample flows through the lysing chamber 86. The captured cells or viruses are then disrupted (lysed) in the chamber 86. The cells may be animal or plant cells, spores, bacteria, or microorganisms. The viruses may be any type of infective agents having a protein coat surrounding an RNA or DNA core.

The filter stack 87 comprises a gasket 93, a first filter 94, a gasket 95, a second filter 97 having a smaller pore size than the first filter 94, a gasket 98, a third filter 100 having a smaller pore size than the second filter 97, a gasket 101, a woven mesh 102, and a gasket 103. The filter stack also preferably includes a first set of beads 96 disposed between the first and second filters 94 and 97 and a second set of beads 99 disposed between the second and third filters 97 and 100. The filter ring 88 compresses the filter stack 87 into the lysing chamber 86 so that the gasket 93 is pressed against the filter 94, the filter 94 is pressed against the gasket 95, the gasket 95 is pressed against the filter 97, the filter 97 is pressed against the gasket 98, the gasket 98 is pressed against the filter 100, the filter 100 is pressed against the gasket 101, the gasket 101 is pressed against the mesh 102, the mesh 102 is pressed against the gasket 103, and the gasket 103 is pressed against the outer perimeter of the bottom wall of the lysing chamber 86. The gasket 95 is thicker than the average diameter of the beads 96 so that the beads are free to move in the space between the filters 94 and 97. Similarly, the gasket 98 is thicker than the average diameter of the beads 99 so that the beads 99 are free to move in the space between the filters 97 and 100. A fluid sample flowing through the channel 106 into the lysing chamber 86 first flows through filter 94, then through filter 97, next through filter 100, and lastly through the

mesh 102. After flowing through the filter stack 87, the sample flows along flow ribs 91 formed in the top of the lysing chamber 86 and through an outlet channel (not shown in Fig. 6).

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Referring to Fig. 5, the cells or viruses captured in the filter stack (not shown in Fig. 5 for illustrative clarity) are lysed by coupling a transducer 92 (e.g., an ultrasonic horn) directly to the wall of the lysing chamber 86. In 10 this embodiment, the wall of the lysing chamber 86 is formed by the flexible gasket 63. The transducer 92 should directly contact an external surface of the wall. The term "external surface" is intended to mean a surface of the wall that is external to the lysing chamber 86. The 15 transducer 92 is a vibrating or oscillating device that is activated to generate pressure waves in the chamber 86. The pressure waves agitate the beads 96, 99 (Fig. 6), and the movement of the beads ruptures the captured cells or viruses. In general, the transducer for contacting the wall 20 of the lysing chamber 86 may be an ultrasonic, piezoelectric, magnetostrictive, or electrostatic transducer. The transducer may also be an electromagnetic device having a wound coil, such as a voice coil motor or a solenoid device. It is presently preferred that the 25 actuator be an ultrasonic transducer, such as an ultrasonic horn. Suitable horns are commercially available from Sonics & Materials, Inc. having an office at 53 Church Hill, Newton, Connecticut 06470-1614 USA. Alternatively, the ultrasonic transducer may comprise a piezoelectric disk or 30 any other type of ultrasonic transducer that may be coupled to the container. It is presently preferred to use an ultrasonic horn because the horn structure is highly resonant and provides for repeatable and sharp frequency of excitation and large motion of the horn tip.

As previously described in Fig. 6, the filter stack includes a gasket at both of its ends. As shown in Fig. 5, the middle cartridge piece 24 has a sealing lip 90 against 5 which the gasket at one end of the filter stack is compressed. The gasket at the other end of the filter stack is compressed by the filter ring 88 to form a seal. The gasket material may expand into the relief area outside of the sealing lip 90. The width of the sealing lip 90 is 10 small (typically 0.5 mm) so that an excessive amount of force is not required to achieve a sufficient seal.

The filter ring 88 is held between the filter stack and the cartridge gasket 63. The cartridge gasket 63 is held 15 between the middle piece 24 and the bottom piece 26 by a sealing lip 406. Force is therefore transferred from the bottom piece 26 through the gasket 63 to the filter ring 88 and finally to the filter stack. The filter ring 88 contains a contact lip 404 that contacts the gasket 63. The 20 contact lip 404 is not a primary sealing lip (though it will seal) but a force transfer mechanism. The width of the contact lip 404 is larger than the width of the sealing lip 90 to ensure that deformation and sealing action occurs in the filter stack and not taken up in squeezing the 25 cartridge gasket 63. The cartridge middle piece 24 also has a sealing lip 406 that surrounds the filter ring 88. This is an active sealing area that should not be compromised by the presence of the filter ring 88. For this reason, there is a gap 407 between the sealing lip 406 and the contact 30 lip 404 on the filter ring 88. The gap 407 is provided to allow the gasket 63 to extrude into the gap 407 as it is compressed by the sealing lip 406 and the contact lip 404. If the contact lip 404 comes to a different elevation than the sealing lip 406, the seal will not be compromised 35 because of the gap 407 and the distance between the lips 404 and 406.

Referring again to Fig. 6, the filter stack 87 is effective for capturing cells or viruses as a fluid sample flows through the stack 87 without clogging of any of the filters 94, 97, 100 in the stack. The first filter 94 (having the largest pore size) filters out coarse material such as salt crystals, cellular debris, hair, tissue, etc. The second filter 97 (having the medium pore size) captures cells or viruses in the fluid sample. The third filter 100 (having the smallest pore size) captures smaller cells or viruses in the sample. The filter stack 87 thus enables the simultaneous capture of differently sized sample components without clogging of the filters. The average pore size of the first filter 94 is selected to be small enough to filter coarse material from the fluid sample (e.g., salt crystals, cellular debris, hair, tissue) yet large enough to allow the passage of the target cells or viruses containing the desired analyte (e.g., nucleic acid or proteins). In general, the pore size of the first filter 94 should be in the range of about 2 to 25  $\mu\text{m}$ , with a presently preferred pore size of about 5  $\mu\text{m}$ .

The average pore sizes of the second and third filters are selected in dependence upon the average size of the target cells or viruses that contain the desired analyte(s). For example, in one embodiment, the filter stack 87 is used to capture gonorrhea (GC) and chlamydia (Ct) organisms to determine the presence of the diseases in the fluid sample. The GC and Ct organisms have different average diameters, about 1 to 2  $\mu\text{m}$  for GC organisms and about 0.3  $\mu\text{m}$  for Ct organisms. In this embodiment, the second filter 97 has an average pore size of about 1.2  $\mu\text{m}$  while the third filter 100 has an average pore size of about 0.22  $\mu\text{m}$  so that most of the GC organisms are captured by the second filter 97 while most of the Ct organisms are captured by the third filter 100. The filter stack thus enables the simultaneous capture of differently sized target organisms and does so without clogging of the filters. The pore sizes of the

filters 97, 100 may be selected to capture desired cells or viruses of any size, and the scope of the invention is not limited to the specific example given.

5 The filter stack 87 is also useful for disrupting the captured cells or viruses to release the intracellular material (e.g., nucleic acid) therefrom. The first and second sets of beads 96, 99 serve two useful purposes in this regard. First, the beads are agitated by the pressure  
10 waves generated by the transducer. The movement of the beads ruptures the captured cells or viruses. Second, the beads may shear the nucleic acid released from the lysed cells or viruses so that the strands of nucleic acid are sufficiently short to flow through the filters and out of  
15 the lysing chamber 86. Suitable beads for rupturing cells or viruses include borosilicate glass, lime glass, silica, and polystyrene beads.

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The beads may be porous or non-porous and preferably have  
20 an average diameter in the range of 1 to 200  $\mu\text{m}$ . The average diameter of the beads 96, 99 is selected in dependence upon the intended target cells or viruses to be ruptured by the beads. The average diameter of the beads 96 in the first set may be equal to the average diameter of  
25 the beads 99 in the second set. Alternatively, when the first set of beads 96 is used to rupture a type of target cell or virus that differs from the type of cell or virus to be ruptured by the second set of beads 99, it is advantageous to select the average diameter of the beads  
30 such that the average diameter of the beads 96 in the first set differs from the average diameter of the beads 99 in the second set. For example, when the filter stack is used to capture GC and Ct cells as described above, the beads 96 are 20  $\mu\text{m}$  diameter borosilicate glass beads for rupturing

the GC organisms and the beads 99 are 106  $\mu\text{m}$  diameter soda lime glass beads for rupturing the Ct organisms. Each of the silicone gaskets 95, 98 should be sufficiently thick to allow room for the beads 96, 99 to move and rupture the  
5 cells or viruses.

The mesh 102 also serves two useful purposes. First the mesh provides support to the filter stack 87. Second, the mesh breaks up air bubbles so that the bubbles can be  
10 channeled through the flow ribs 91 and out of the lysing chamber 86. To effectively break up or reduce the size of the air bubbles, the mesh 102 preferably has a small pore size. Preferably, it is a woven polypropylene mesh having an average pore size of about 25  $\mu\text{m}$ . To ensure that the air  
15 bubbles can escape from the lysing chamber 86, it is desirable to use the cartridge in an orientation in which liquid flows up (relative to gravity) through the filter stack 87 and the lysing chamber 86. The upward flow through the chamber 86 aids the flow of air bubbles out of the  
20 chamber 86. Thus, the inlet port for entry of fluids into the chamber 86 should generally be at the lowest point in the chamber, while the exit port should be at the highest.

Many different embodiments of the filter stack are  
25 possible. For example, in one alternative embodiment, the filter stack has only two filters and one set of beads disposed between the filters. The first filter has the largest pore size (e.g., 5  $\mu\text{m}$ ) and filters out coarse material such as salt crystals, cellular debris, hair,  
30 tissue, etc. The second filter has a pore size smaller than the first filter and slightly smaller than the target cells or viruses to be captured. Such a filter stack is described below with reference to Fig. 38. In another embodiment of the cartridge, the filter having the largest  
35 pore size (for filtering the coarse material) is positioned

in a filter chamber (not shown) that is positioned upstream of the lysing chamber 86. A channel connects to the filter chamber to the lysing chamber 86. In this embodiment, a fluid sample flows first through the coarse filter in the 5 filter chamber and then through a second filter in the lysing chamber to trap the target cells or viruses in the lysing chamber.

Further, the beads in the filter stack may have a binding 10 affinity for target cells or viruses in the fluid sample to facilitate capture of the target cells or viruses. For example, antibodies or certain receptors may be coated onto the surface of the beads to bind target cells in the sample. Moreover, the lysing chamber 86 may contain two 15 different types of beads for interacting with target cells or viruses. For example, the lysing chamber may contain a first set of beads coated with antibodies or receptors for binding target cells or viruses and a second set of beads (intermixed with the first set) for rupturing the captured 20 cells or viruses. The beads in the lysing chamber 86 may also have a binding affinity for the intracellular material (e.g., nucleic acid) released from the ruptured cells or viruses. Such beads are useful for isolating target nucleic acid for subsequent elution and analysis. For example, the 25 lysing chamber may contain silica beads to isolate DNA or cellulose beads with oligo dT to isolate messenger RNA for RT-PCR. The lysing chamber 86 may also contain beads for removing unwanted material (e.g., proteins, peptides) or chemicals (e.g., salts, metal ions, or detergents) from the 30 sample that might inhibit PCR. For example, the chamber 86 may contain ion exchange beads for removing proteins. Alternatively beads having metal ion chelators such as iminodiacetic acid will remove metal ions from biological samples.

Figs. 21-22 illustrate the reaction vessel 40 in greater detail. Fig. 21 shows a partially exploded view of the vessel 40, and Fig. 22 shows a front view of the vessel 40.

5 The vessel 40 includes the reaction chamber 42 (diamond-shaped in this embodiment) for holding a reaction mixture. The vessel 40 is designed for optimal heat transfer to and from the reaction mixture and for efficient optical viewing of the mixture. The thin shape of the vessel contributes to  
10 optimal thermal kinetics by providing large surfaces for thermal conduction and for contacting thermal plates. In addition, the walls of the vessel provide optical windows into the chamber 42 so that the entire reaction mixture can be optically interrogated. In more detail to Figs. 21-22,  
15 the reaction vessel 40 includes a rigid frame 46 that defines the side walls 57A, 57B, 59A, 59B of the reaction chamber 42. The frame 46 also defines an inlet port 41 and a channel 50 connecting the port 41 to the chamber 42. The frame 46 also defines an outlet port 43 and a channel 52  
20 connecting the port 43 to the chamber 42. The inlet port 41 and channel 50 are used to add fluid to the chamber 42, and the channel 52 and outlet port 43 are used for exit of fluid from the chamber 42. Alignment prongs 44A, 44B are used to position the vessel 40 correctly during assembly of  
25 the cartridge.

As shown in Fig. 21, the vessel 40 also includes thin, flexible sheets attached to opposite sides of the rigid frame 46 to form opposing major walls 48 of the chamber.

30 (The major walls 48 are shown in Fig. 1 exploded from the rigid frame 46 for illustrative clarity). The reaction chamber 42 is thus defined by the rigid side walls 57A, 57B, 59A, 59B of the frame 46 and by the opposing major walls 48. The opposing major walls 48 are sealed to

opposite sides of the frame 46 such that the side walls 57A, 57B, 59A, 59B connect the major walls 48 to each other. The walls 48 facilitate optimal thermal conductance to the reaction mixture contained in the chamber 42. Each 5 of the walls 48 is sufficiently flexible to contact and conform to a respective thermal surface, thus providing for optimal thermal contact and heat transfer between the thermal surface and the reaction mixture contained in the chamber 42. Furthermore, the flexible walls 48 continue to 10 conform to the thermal surfaces if the shape of the surfaces changes due to thermal expansion or contraction during the course of the heat-exchanging operation.

As shown in Fig. 23, the thermal surfaces for contacting 15 the flexible walls 48 are preferably formed by a pair of opposing plates 190A, 190B positioned to receive the chamber 42 between them. When the chamber 42 of the vessel 40 is inserted between the plates 190A, 190B, the inner surfaces of the plates contact the walls 48 and the 20 flexible walls conform to the surfaces of the plates. The plates are preferably spaced a distance from each other equal to the thickness T of the chamber 42 as defined by the thickness of the frame 46. In this position, minimal or no gaps are found between the plate surfaces and the walls 25 48. The plates may be heated and cooled by various thermal elements to induce temperature changes within the chamber 42, as is described in greater detail below.

The walls 48 are preferably flexible films of polymeric 30 material such as polypropylene, polyethylene, polyester, or other polymers. The films may either be layered, e.g., laminates, or the films may be homogeneous. Layered films are preferred because they generally have better strength and structural integrity than homogeneous films. In

particular, layered polypropylene films are presently preferred because polypropylene is not inhibitory to PCR. Alternatively, the walls 48 may comprise any other material that may be formed into a thin, flexible sheet and that

5 permits rapid heat transfer. For good thermal conductance, the thickness of each wall 48 is preferably between about 0.003 to 0.5 mm, more preferably between 0.01 to 0.15 mm, and most preferably between 0.025 to 0.08 mm.

10 Referring again to Fig. 22, the vessel 40 also preferably includes optical windows for *in situ* optical interrogation of the reaction mixture in the chamber 42. In the preferred embodiment, the optical windows are the side walls 57A, 57B of the rigid frame 46. The side walls 57A, 57B are

15 optically transmissive to permit excitation of the reaction mixture in the chamber 42 through the side wall 57A and detection of light emitted from the chamber 42 through the side wall 57B. Arrows A represent illumination beams entering the chamber 42 through the side wall 57A and

20 arrows B represent emitted light (e.g., fluorescent emission from labeled analytes in the reaction mixture) exiting the chamber 42 through the side wall 57B.

The side walls 57A, 57B are preferably angularly offset

25 from each other. It is usually preferred that the walls 57A, 57B are offset from each other by an angle of about 90°. A 90° angle between excitation and detection paths assures that a minimum amount of excitation radiation entering through the wall 57A will exit through wall 57B.

30 In addition, the 90° angle permits a maximum amount of emitted light (e.g. fluorescence) to be collected through wall 57B. The walls 57A, 57B are preferably joined to each other to form a "V" shaped intersection at the bottom of the chamber 42. Alternatively, the angled walls 57A, 57B

need not be directly joined to each other, but may be separated by an intermediary portion, such as another wall or various mechanical or fluidic features which do not interfere with the thermal and optical performance of the vessel. For example, the walls 57A, 57B may meet at a port which leads to another processing area in communication with the chamber 42, such as an integrated capillary electrophoresis area. In the presently preferred embodiment, a locating tab 58 extends from the frame 46 below the intersection of walls 57A, 57B. The tab 58 is used to properly position the vessel 40 in a heat-exchanging module described below with reference to Fig. 28.

Optimum optical sensitivity may be attained by maximizing the optical path length of the light beams exciting the labeled analyte in the reaction mixture and the emitted light that is detected, as represented by the equation:

$$I_o/I_i = C * L * A,$$

where  $I_o$  is the illumination output of the emitted light in volts, photons or the like,  $C$  is the concentration of analyte to be detected,  $I_i$  is the input illumination,  $L$  is the path length, and  $A$  is the intrinsic absorptivity of the dye used to label the analyte.

The thin, flat reaction vessel 40 of the present invention optimizes detection sensitivity by providing maximum optical path length per unit analyte volume. Referring to Figs. 23 and 27, the vessel 40 is preferably constructed such that each of the side walls 57A, 57B, 59A, 59B of the chamber 42 has a length  $L$  in the range of 1 to 15 mm, the chamber has a width  $W$  in the range of 1.4 to 20 mm, the chamber has a thickness  $T$  in the range of 0.5 to 5mm, and the ratio of the width  $W$  of the chamber to the thickness  $T$

of the chamber is at least 2:1. These parameters are presently preferred to provide a vessel having a relatively large average optical path length through the chamber, i.e. 1 to 15 mm on average, while still keeping the chamber 5 sufficiently thin to allow for extremely rapid heating and cooling of the reaction mixture contained therein. The average optical path length of the chamber 42 is the distance from the center of the side wall 57A to the center of the chamber 42 plus the distance from the center of the 10 chamber 42 to the center of the side wall 57B.

More preferably, the vessel 40 is constructed such that each of the sides walls 57A, 57B, 59A, 59B of the chamber 42 has a length L in the range of 5 to 12 mm, the chamber 15 has a width W in the range of 7 to 17 mm, the chamber has a thickness T in the range of 0.5 to 2 mm, and the ratio of the width W of the chamber to the thickness T of the chamber is at least 4:1. These ranges are more preferable because they provide a vessel having both a larger average 20 optical path length (i.e., 5 to 12 mm) and a volume capacity in the range of 12 to 100  $\mu$ l while still maintaining a chamber sufficiently thin to permit extremely rapid heating and cooling of a reaction mixture. The relatively large volume capacity provides for increased 25 sensitivity in the detection of low concentration analytes, such as nucleic acids.

In the preferred embodiment, the reaction vessel 40 has a diamond-shaped chamber 42 defined by the side walls 57A, 30 57B, 59A, 59B, each of the side walls has a length of about 10 mm, the chamber has a width of about 14 mm, the chamber has a thickness T of 1 mm as defined by the thickness of the frame 46, and the chamber has a volume capacity of about 100  $\mu$ l. This reaction vessel provides a relatively

large average optical path length of 10 mm through the chamber 42. Additionally, the thin chamber allows for extremely rapid heating and/or cooling of the reaction mixture contained therein. The diamond-shape of the chamber 42 helps prevent air bubbles from forming in the chamber as it is filled with the reaction mixture and also aids in optical interrogation of the mixture.

Referring again to Fig. 22, the frame 46 is preferably made of an optically transmissive material, e.g., a polycarbonate or clarified polypropylene, so that the side walls 57A, 57B are optically transmissive. As used herein, the term optically transmissive means that one or more wavelengths of light may be transmitted through the walls.

In the preferred embodiment, the optically transmissive walls 57A, 57B are substantially transparent. In addition, one or more optical elements may be present on the optically transmissive side walls 57A, 57B. The optical elements may be designed, for example, to maximize the total volume of solution which is illuminated by a light source, to focus excitation light on a specific region of the chamber 42, or to collect as much fluorescence signal from as large a fraction of the chamber volume as possible.

In alternative embodiments, the optical elements may comprise gratings for selecting specific wavelengths, filters for allowing only certain wavelengths to pass, or colored lenses to provide filtering functions. The wall surfaces may be coated or comprise materials such as liquid crystal for augmenting the absorption of certain wavelengths. In the presently preferred embodiment, the optically transmissive walls 57A, 57B are substantially clear, flat windows having a thickness of about 1 mm.

The side walls 59A, 59B preferably includes reflective faces 56 which internally reflect light trying to exit the chamber 42 through the side walls 59A, 59B. The reflective faces 56 are arranged such that adjacent faces are

5 angularly offset from each other by about 90°. In addition, the frame 46 defines open spaces between the side walls 59A, 59B and the support ribs 53. The open spaces are occupied by ambient air that has a different refractive index than the material composing the frame (e.g.,

10 plastic). Due to the difference in the refractive indexes, the reflective faces 56 are effective for internally reflecting light trying to exit the chamber through the walls 59A, 59B and provide for increased detection of optical signal through the walls 57A, 57B. Preferably, the

15 optically transmissive side walls 57A, 57B define the bottom portion of the diamond-shaped chamber 42, and the retro-reflective side walls 59A, 59B define the top portion of the chamber.

20 A preferred method for fabricating the reaction vessel 40 will now be described with reference to Figs. 21-22. The reaction vessel 40 may be fabricated by first molding the rigid frame 46 using known injection molding techniques. The frame 46 is preferably molded as a single piece of

25 polymeric material, e.g., clarified polypropylene. After the frame 46 is produced, thin, flexible sheets are cut to size and sealed to opposite sides of the frame 46 to form the major walls 48 of the chamber 42. The major walls 48 are preferably cast or extruded films of polymeric

30 material, e.g., polypropylene films, that are cut to size and attached to the frame 46 using the following procedure. A first piece of film is placed over one side of the frame 46. The frame 46 preferably includes a tack bar 47 for aligning the top edge of the film. The film is placed over

the bottom portion of the frame 46 such that the top edge of the film is aligned with the tack bar 47 and such that the film completely covers the bottom portion of the frame 46 below the tack bar 47. The film should be larger than  
5 the bottom portion of the frame 46 so that it may be easily held and stretched flat across the frame. The film is then cut to size to match the outline of the frame by clamping to the frame the portion of the film that covers the frame and cutting away the portions of the film that extend past  
10 the perimeter of the frame using, e.g., a laser or die.  
The film is then tack welded to the frame, preferably using a laser.

The film is then sealed to the frame 46, preferably by heat  
15 sealing. Heat sealing is presently preferred because it produces a strong seal without introducing potential contaminants to the vessel as the use of adhesive or solvent bonding techniques might do. Heat sealing is also simple and inexpensive. The heat sealing may be performed  
20 using, e.g., a heated platen. An identical procedure may be used to cut and seal a second sheet to the opposite side of the frame 46 to complete the chamber 42. Many variations to this fabrication procedure are possible. For example, in an alternative embodiment, the film is stretched across  
25 the bottom portion of the frame 46 and then sealed to the frame prior to cutting the film to size. After sealing the film to the frame, the portions of the film that extend past the perimeter of the frame are cut away using, e.g., a laser or die.

30

Although it is presently preferred to mold the frame 46 as a single piece, it is also possible to fabricate the frame from multiple pieces. For example, the side walls 57A, 57B forming the angled optical windows may be molded from

polycarbonate, which has good optical transparency, while the rest of the frame is molded from polypropylene, which is inexpensive and compatible with PCR. The separate pieces can be attached together in a secondary step. For example,

5 the side walls 57A, 57B may be press-fitted and/or bonded to the remaining portion of the frame 46. The flexible walls 48 may then be attached to opposite sides of the frame 46 as previously described.

10 Referring again to Fig. 3, it is presently preferred to use a gasket 61 to seal the ports 41, 43 of the vessel 40 to corresponding channels 80, 81 (Fig. 4) in the cartridge body. Alternatively, fluidic seals may be established using a luer fitting, compression fitting, or swaged fitting. In

15 another embodiment, the cartridge body and frame of the vessel 40 are molded as a single part, and the flexible major walls of the vessel are heat-sealed to opposite sides of the frame.

20 Referring again to Fig. 22, the chamber 42 is filled by forcing liquid (e.g., a reaction mixture) to flow through the port 41 and the channel 50 into the chamber 42. The liquid may be forced to flow into the chamber 42 using differential pressure (i.e., either pushing the liquid

25 through the inlet port 41 or aspirating the liquid by applying a vacuum to the outlet port 43). As the liquid fills the chamber 42, it displaces air in the chamber. The displaced air exits the chamber 42 through the channel 52 and the port 43. For optimal detection of analyte in the

30 chamber 42, the chamber should not contain air bubbles. To help prevent the trapping of air bubbles in the chamber 42, the connection between the chamber 42 and the outlet channel 52 should be at the highest point (with respect to gravity) in the chamber 42. This allows air bubbles in the

35 chamber 42 to escape without being trapped. Thus, the

vessel 40 is designed to be used in the vertical orientation shown in Fig. 22.

Fig. 25 shows another vessel 206 designed to be used in a horizontal orientation. The vessel 206 has an inlet port 41 and an inlet channel 50 connecting the inlet port 41 to the bottom of the chamber 42. The vessel also has an outlet port 43 and an outlet channel 50 connecting the outlet port 43 to the top of the chamber 42. Thus, any air bubbles in the chamber 42 may escape through the outlet channel 52 without becoming trapped. Fig. 26 shows another vessel 207 having two inlet ports 41, 45 and one outlet port 43. Inlet channels 50, 54 connect the respective inlet ports 41, 45 to the chamber 42, and outlet channel 52 connects the chamber 42 to outlet port 43. Many other different embodiments of the vessel are also possible. In each embodiment, it is desirable to evacuate the chamber 42 from the highest point (with respect to gravity) in the chamber and to introduce liquid into the chamber from a lower point.

Figs. 15A-15B illustrate two types of valves used in the cartridge. As shown in Fig. 15A, there are two types of fundamental concepts to the valve action, and hence two types of valves. The first valve uses a cone-shaped or conical valve seat 160 formed in the middle cartridge piece 24. The valve seat 160 is a depression, recess, or cavity molded or machined in the middle piece 24. The valve seat 160 is in fluid communication with a chamber 167 through a port or channel 157 that intersects the center of the conical valve seat 160. As shown in Fig. 15B, a valve actuator 164 having a spherical surface is forced against the elastic membrane 63 and into the valve seat 160, establishing a circular ring of contact between the membrane 63 and the valve seat 160. The kinematic principle is that of a ball seated into a cone. The circular seal

formed by the membrane 63 and valve seat 160 prevents flow between the channel 157 (and hence the chamber 167) and a side channel 158 extending from a side of the valve seat 160. The side channel 158 is defined by the membrane 63 and 5 the middle cartridge piece 24.

As shown in Fig. 15A, the other type of valve controls the cross flow between the channel 158 and another side channel 159 formed between the membrane 63 and the middle cartridge 10 piece 24. In this case, a circular ring of contact would be ineffective. Instead, the second valve comprises a recess depression or cavity 161 formed in the middle cartridge piece 24. The cavity 161 separates the channels 158, 159 from each other. An end of the channel 158 is positioned on 15 one side of the cavity 161, and an end of the channel 159 is positioned on the opposite side of the cavity 161. The cavity 161 is defined by a first curved surface 162A positioned adjacent the end of the channel 158, a second curved surface 162B positioned adjacent the end of the channel 159, and a third surface 163 between the first and 20 second curved surfaces 162A, 162B. As shown in Fig. 15B, the curved surfaces provide two valve seats that are the primary contact area for the membrane 63 to seal off the flow between the channels 158 and 159. The kinematic principle is that of a ball (or spherical end on a valve actuator) held by three contact points, the upward force on 25 the actuator and the two valve seats 162A, 162B.

As shown in Fig. 16A, the first and second curved surfaces 30 162A, 162B are preferably concentric spherical surfaces. The valve actuator 164 has also has a spherical surface for pressing the membrane 63 tightly against the surfaces 162A, 162B. In addition, each of the surfaces 162A, 162B preferably has a spherical radius of curvature R1 equal to 35 the combined radius of curvature R2 of the valve actuator 164 plus the thickness T of the membrane 63. For example, if the radius of curvature R2 of the surface of the valve actuator 164 is .094 inches and the membrane 63 has a

thickness  $T$  of 0.031 inches, then the radius of curvature  $R_1$  of each of the surfaces 162A, 162B is 0.125 inches. In general, the size and radius of curvature of the valve seats is dependent upon the size of the channels in the 5 cartridge. The valves are preferably made just large enough to effectively seal the channels but no larger so that dead volume in the cartridge is minimized.

As shown in Fig. 16B, the third surface 163 is recessed 10 from the first and second surfaces 162A, 162B to provide a gap 166 between the membrane 63 and the third surface 163 when the membrane 63 is pressed against the first and second surfaces 162A, 162B. Stated another way, the surfaces 162A, 162B are raised or elevated from the surface 15 163. The gap 166 ensures that the membrane 63 contacts primarily the valve seats 162A, 162B rather than the entire surface of the cavity 161 so that maximum pressure is applied to the valve seats 162A and 162B by the membrane 63. This provides a very strong seal with minimal actuator 20 force required.

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Referring again to Fig. 15B, in both types of valves the respective kinematic principle defines the location of the mating parts. In both the ball-in-cone concept and the 25 ball-against-two-spherical-surfaces concept, the ball or spherical shaped valve actuator is permitted to seek its own location as it is forced against the valve seat(s). There is a deliberate clearance (e.g., 0.01 to 0.03 inches) between the valve actuator and the hole in the bottom 30 cartridge piece 26 in which the actuator 164 travels so that only the valve seat action defines the location of the mating pieces.

The valve actuators can be controlled by a variety of 35 mechanisms. Figs. 17-19 illustrate one such mechanism. As shown in Fig. 17, a valve actuator 172 has a spherical surface for pressing the gasket 63 into a valve seat. The actuator 172 also has a flange 177 on its bottom portion.

The cartridge includes an elastic body, such as a spring 174, that pushes against a ledge in the lower cartridge piece 26 to bias the valve actuator against the gasket 63. The spring 174 is sufficiently strong to close the valve  
5 unless a deliberate force is applied to pull down the actuator 172. The valves in the cartridge may be kept closed in this manner for shipping and storage before the cartridge is used. Thus, the cartridge may be preloaded during manufacture with the necessary reagents and wash  
10 solutions to analyze a fluid sample without the fluids leaking out of the cartridge during shipping and storage.

The actuator pull-down mechanism is usually located in an instrument into which the cartridge is placed for sample  
15 analysis (one such instrument is described in detail below with reference to Fig. 10). The mechanism comprises a sliding guide 175 that rotates a hinged pull-down member 180 having a jaw 181 for receiving the flange 177 of the actuator 172. As shown in Fig. 18, the sliding guide 175  
20 rotates the hinged pull-down member 180 until the flange 177 is positioned within the jaw 181. As shown in Fig. 19, a solenoid 146 pulls down the member 180 and thus the valve actuator 172 so that the gasket 63 is released from the valve seat, thus opening the valve and permitting fluid  
25 flow between the channels 170 and 171.

Fig. 20 illustrates the manner in which fluid flow into and out of the sample chamber, wash chamber, neutralizer chamber, and reagent chambers is controlled in the  
30 cartridge. Each of these chambers, as illustrated by a chamber 414 in Fig. 20, is covered by a hydrophobic membrane 410 that allows the passage of gas but not liquid therethrough. The hydrophobic membrane 410 is positioned between the chamber 414 and a pressure port 32. The  
35 pressure port 32 is formed in the upper cartridge piece 22 and positioned over the chamber 414. The membrane 410 holds liquids in the chamber 414 during shipping and storage of the cartridge, even if the cartridge is turned upside down.

The pressure port 32 is sized to receive a pressure nozzle 182 that is connected to a pressure source (e.g., a vacuum or pneumatic pump) usually located in the external instrument. The nozzle 182 includes an o-ring 184 and a flange 415. A spring 185 pushes against the flange 415 to force the nozzle 182 into the pressure port 32 so that the o-ring 184 establishes a seal around the port 32. In operation, positive air pressure or a vacuum is applied to the chamber 414 through the pressure port 32 to force liquids out of or into, respectively, the chamber 414.

A conical valve seat 160 (previously described with reference to Figs. 15A-15B) is formed in the middle cartridge piece 24 below the chamber 414 to control the flow of liquid between the chamber 414 and a connecting channel 411. The valve is opened and closed by a valve actuator 188 having a flange 187 and a spring 188 pressing against the flange to hold the valve closed until a downward force is applied to the actuator 186. The downward force is preferably supplied by a solenoid that pulls down the actuator 186 to open the valve. The valve actuator 186 and solenoid are preferably located in the instrument.

Figs. 7-8 show top and bottom plan views, respectively, of the cartridge. Fig. 9 is a schematic block diagram of the cartridge. As shown in any of Figs. 7-9, the cartridge includes a sample chamber 65 having a port for adding a fluid sample to the cartridge and a sample flow path extending from the sample chamber 65. The sample flow path extends from the sample chamber 65 through a valve 107 and into a channel 106. The channel 106 includes a sensor region 136 in which the channel 106 has a flat bottom enabling easy optical detection of the presence of liquid in the channel. The sample flow path continues from the channel 106 into the lysing chamber 86 and through the filter stack 87. The sample flow path also includes a channel 109 for exit of fluid from the lysing chamber 86, a

channel 110 having a flat-bottomed detection region 137, a valve 111, and a channel 112 leading to the vented waste chamber 68 through a valve 114.

5 The cartridge also includes the wash chamber 66 for holding wash solution and the reagent chamber 67 for holding lysing reagent. The wash chamber 66 is connected to the lysing chamber 86 through a valve 115, channel 117, and channel 106. The reagent chamber 67 is connected to the lysing  
10 chamber 86 through a valve 119, channel 117, and channel 106. Sample components (e.g., cells or viruses in the sample) are captured in the filter stack 87 and lysed in the chamber 86 to release target analyte (e.g., nucleic acid) from the sample components. The cartridge also  
15 includes an analyte flow path extending from the lysing chamber 86 for carrying the analyte separated from the fluid sample to the reaction vessel 40 for chemical reaction and optical detection. The analyte flow path extends from the chamber 86 through the channel 109,  
20 channel 110, and valve 111. After passing through the valve 111, the analyte flow path diverges from the sample flow path. While the sample flow path extends though channel 112 into the waste chamber 68, the analyte flow path diverges into the U-shaped channel 122. The analyte  
25 flow path then extends into and out of the neutralizer chamber 70 through a valve 124. The analyte flow path also passes into and out of the master mix chamber 71 through a valve 126. From the master mix chamber 71, the analyte flow path extends along the channel 122, through a valve  
30 127, through channel 80, and into the reaction vessel 40 through the port 41.

The reaction vessel 40 includes the port 41 for adding a reaction mixture to the vessel, and the port 43 for exit of  
35 fluids (e.g., air or excess reaction mixture) from the vessel. The cartridge also includes channel 81 in fluid communication with the port 43. The channel 81 includes a flat-bottomed detection region 130 for detecting the

presence of liquid in the channel. The channel 81 connects to a channel 131 (channel 131 extends straight down perpendicular to the page in the top plan view of Fig. 7). Channel 131 connects to a channel 132 which in turn 5 connects to a channel 134 through a valve 133 (channel 134 extends straight up perpendicular to the page in the top plan view of Fig. 7). The channel 134 leads to the vent 36 which has a hydrophobic membrane to permit the escape of gas but not liquid from the cartridge. The channels, vent 10 and valve positioned downstream from the reaction vessel 40 are used to pressurize the chamber 42 of the vessel, as is described in the operation section below.

The cartridge also includes a first pressure port 105 15 positioned above the sample chamber 65, a second pressure port 116 positioned above the wash chamber 66, a third pressure port 118 positioned above the reagent chamber 67, a fourth pressure port 123 positioned above the neutralizer chamber 70, a fifth pressure port 125 positioned above the 20 master mix chamber 71, and a sixth pressure port 128 positioned at the end of the U-shaped channel 122. The cartridge further includes sensor chambers 120 and 121 in fluid communication with the waste chamber 68. The sensor chambers 120 and 121 indicate when predetermined volumes of 25 liquid have been received in the waste chamber 68, as is described in detail below.

Referring to Fig. 10, the cartridge is preferably used in combination with an instrument 140 designed to accept one 30 or more of the cartridges. For clarity of illustration, the instrument 140 shown in Fig. 10 accepts just one cartridge. It is to be understood, however, that the instrument may be designed to process multiple cartridges simultaneously. The instrument 140 includes a cartridge 35 nest 141 into which the cartridge is placed for processing. The instrument 140 also includes the transducer 92 (e.g., an ultrasonic horn) for generating pressure waves in the lysing chamber of the cartridge, nine valve actuators 142

for actuating the nine valves in the cartridge, nine corresponding solenoids 146 for pulling down the valve actuators, and six pressure nozzles 145 for interfacing with six corresponding pressure ports formed in the 5 cartridge. In addition, the instrument includes or is connected to one or more regulated pressure sources for supplying pressure to the cartridge through the pressure nozzles 145. Suitable pressure sources include syringe pumps, compressed air sources, pneumatic pumps, or 10 connections to external sources of pressure. The instrument further includes three slotted optical sensors 143 and three reflective optical sensors 144.

Fig. 13 illustrates the slotted optical sensors 143 15 positioned to detect liquid in the sensor chambers 120, 121 and in the reagent chamber 67. Each sensor 143 includes a built in LED and photodiode positioned on opposite sides of the sensor. The LED emits a beam that is detected by the photodiode if the beam is not substantially refracted. 20 Such slotted optical sensors are commercially available from a number of suppliers. The cartridge is shaped so that the slotted optical sensors fit around the chambers 67, 120, and 121. The operation of each sensor is as follows. If liquid is not present in the chamber the 25 sensor surrounds, the beam from the LED is substantially refracted by air in the chamber and the curved inner walls of the chamber and only a weak signal, if any, is detected by the photodiode since air has an index of refraction that does not closely match that of the plastic cartridge. If 30 there is liquid present in the chamber, however, the beam from the LED does not refract or is only slightly refracted and produces a much stronger signal detected by the photodiode since the liquid has an index of refraction closely matching that of the plastic cartridge. The 35 optical sensors 143 are therefore useful for determining the presence or absence of liquid in the chambers 67, 120, and 121.

Fig. 14 shows a cut-away, schematic side view of the sensor chamber 120 in fluid communication with the waste chamber 68 and surrounded by the slotted optical sensor 143. The 5 sensor chamber 120 and sensor 143 are used to indicate when a predetermined volume of liquid is present in the waste chamber 68. The sensor chamber 120 is partially separated from the waste chamber 68 by a wall 151 having a spillover rim 152. The height of the wall is selected so that when 10 the predetermined volume of liquid is received in the waste chamber 68, the liquid spills over the spillover rim 152 and into the sensor chamber 120. The liquid in the sensor chamber 120 is then detected by the sensor 143.

15 Referring again to Fig. 13, the cartridge may also include a second sensor chamber 121 in fluid communication with the waste chamber 68. The second sensor chamber 121 is also separated from the waste chamber 68 by a wall 153 having a spillover rim. The wall 153 is taller than the wall 152 so 20 that liquid does not spill over the wall 153 until a second predetermined volume of fluid in addition to the first predetermined volume of fluid has been received in the waste chamber 68. The sensor chambers 120, 121 and the optical sensors 143 are useful for controlling the 25 operation of the cartridge. The height of the wall 152 is preferably selected such that when a fixed volume of fluid sample from the sample chamber 65 has flowed through the sample flow path to the waste chamber 68, the sample liquid spills over into the sensor chamber 120 and is detected.

30 The detection in chamber 120 triggers the release of wash solution from the wash chamber 66 which flows through the sample flow path to the waste chamber 68. When an incremental volume of the wash solution is received in the chamber 68, liquid spills over the wall 153 into the sensor chamber 121 and is detected. The detection of liquid in 35 the chamber 121 then triggers the release of lysing reagent from the chamber 67. The sensor 143 surrounding the chamber 67 may then be used to indicate when the chamber 67 is

empty, triggering the start of ultrasonic lysis. In an alternative embodiment, the cartridge may have two waste chambers, one for sample and one for wash, with each waste chamber having a respective sensor chamber connected  
5 thereto.

In-line reflective optical sensors 144 are used to determine the presence or absence of liquid in the flat-bottomed detection regions 130, 136, 137, of channels 81, 10 106, and 110, respectively (Fig. 7). Each sensor 144 has a built in emitter and detector that is positioned over a flat-bottomed detection region. The emitter emits a beam that is reflected from the cartridge and detected by the detector. The sensor detects a change in signal when an  
15 air/liquid interface passes through the detection region. Optionally, dual emitter reflective optical sensors may be used for a more reliable detection operation. Both types of reflective optical sensors are well known in the art and commercially available.

20 Referring again to Fig. 10, the instrument 140 also includes a heat-exchanging module 147 having a slot 148 for receiving the reaction vessel of the cartridge. The module 147 is described in detail below with reference to Fig. 28.  
25 The instrument 140 further includes a latch mechanism 149 for latching a lid 150 over a cartridge. The cartridge nest 141 includes alignment holes 401 for receiving the legs of the cartridge. The alignment holes 401 ensure proper positioning of the cartridge in the nest 141 so that  
30 the pressure nozzles 145, transducer 92, and valve actuators 142 fit into the corresponding ports in the cartridge and so that the reaction vessel fits into the slot 148. The transducer 92 should be positioned in the instrument 140 such that when the cartridge is placed in  
35 the nest 141, the transducer contacts the bottom wall of the lysing chamber 86, as shown in the cut-away view of Fig. 5. In addition, the instrument may include a spring or similar mechanism to bias the transducer 92 against the

wall of the lysing chamber 86.

The instrument 140 also includes various conventional equipment not shown in Fig. 10 including a main logic board 5 having a microcontroller for controlling the operation of the solenoids 146, transducer 92, heat-exchanging module 147, and optical sensors 143, 144. The instrument also includes or is connected to a power supply for powering the instrument and a pneumatic pump for supplying air pressure 10 through the nozzles 145. The instrument 140 is preferably computer-controlled using, e.g., the microcontroller which is programmed to perform the functions described in the operation section below. Alternatively, the instrument may be controlled by a separate computer, or controlled by a 15 combination of a separate computer and an on-board microcontroller.

Fig. 11 shows an isometric view of the cartridge 20 placed in the instrument 140 for processing. Fig. 11 shows a 20 partial cut-away view of the instrument 140 with the lid 150 closed. Referring again to Fig. 11, a memory or microprocessor chip may optionally be incorporated as part of the cartridge 20. This chip preferably contains information such as the type of cartridge, program 25 information such as specific protocols for the processing of the cartridge, tolerances for accept and reject, serial numbers and lot codes for quality tracking, and provision for storing the results of the processing. Integrated electronic memory on the cartridge 20 allows for rapid, 30 easy, and error-free set-up of the instrument 140 for different fluidic processing protocols. When the cartridge 20 is inserted into the instrument 140, the instrument may electronically address the memory on the cartridge, and thus automatically receive the appropriate set of 35 instructions for controlling the time-sequence of fluidic operations to be carried out with the inserted cartridge. The instrument 140 may simply sequentially retrieve and execute each step in the cartridge's memory, or download

its contents so that the user may edit the sequence using, e.g., the controller computer.

If suitable memory is included on the cartridge, such as  
5 writable memory (e.g., erasable programmable read-only  
memory (EPROM), electrically erasable programmable read-  
only memory (EEPROM), etc., intermediate and final results,  
based on the sample introduced into the cartridge, could be  
written by the instrument into the cartridge's memory for  
10 co-located storage with the physical sample after  
processing. This is particularly advantageous in  
applications where archiving of samples and results is  
necessary, such as forensics. In addition, other  
information can be stored in the memory on the cartridge,  
15 in unalterable (or alterable) forms. For example,  
cartridge serial number, lot manufacture information, and  
related information could be pre-programmed and  
unalterable. User data, technician identification number,  
date of test, location of test and instrument serial number  
20 could be unalterably written into the cartridge. This  
allows for easy identification of the "chain of custody" in  
the handling of a specimen. Engineers skilled in the art  
of data storage will recognize that other memory means than  
electronic can be used, such as optically-addressed printed  
25 regions (e.g., ink-jet or thermal), magnetic strips, etc.

Fig. 28 shows the heat-exchanging module 147 of the  
instrument into which the reaction vessel 40 is inserted  
for thermal processing and optical detection of target  
30 analyte(s) in the reaction mixture. The module 147  
preferably includes a housing 208 for holding the various  
components of the module. The module 147 also includes the  
thermal plates 190 described above. The housing 208  
includes a slot (not shown in Fig. 28) above the plates 190  
35 so that the reaction chamber of the vessel 40 may be  
inserted through the slot and between the plates. The heat-  
exchanging module 147 also preferably includes a cooling

system, such as a fan 212. The fan 212 is positioned to blow cooling air past the surfaces of the plates 190 to cool the plates and hence cool the reaction mixture in the vessel 40. The housing 208 preferably defines channels for 5 directing the cooling air past the plates 190 and out of the module 147.

The heat-exchanging module 147 further includes an optical excitation assembly 216 and an optical detection assembly 10 218 for optically interrogating the reaction mixture contained in the vessel 40. The excitation assembly 216 includes a first circuit board 220 for holding its electronic components, and the detection assembly 216 includes a second circuit board 222 for holding its 15 electronic components. The excitation assembly 216 includes one or more light sources (e.g., an LED, laser, or light bulb) for exciting fluorescently-labeled analytes in the vessel 40. The excitation assembly 216 also includes one or more lenses for collimating the light from the light 20 sources, as well as filters for selecting the excitation wavelength ranges of interest. The detection assembly 218 includes one or more detectors (e.g., a photodiode, photomultiplier tube, or CCD) for detecting the light emitted from the vessel 40. The detection assembly 218 also 25 includes one or more lenses for focusing and collimating the emitted light, as well as filters for selecting the emission wavelength ranges of interest. Suitable optical excitation and detection assemblies for use in the heat-exchanging module 147 are described in International 30 Publication Number WO 99/60380 (International Application Number PCT/US99/11182) published November 25, 1999, the disclosure of which is incorporated by reference herein.

The optics assemblies 216, 218 are positioned in the housing 208 such that when the chamber of the vessel 40 is inserted between the plates 190, the excitation assembly 216 is in optical communication with the chamber 42 through 5 the optically transmissive side wall 57A (see Fig. 22) and the detection assembly 218 is in optical communication with the chamber through the optically transmissive side wall 57B (Fig. 22). In the preferred embodiment, the optics assemblies 216, 218 are placed into optical communication 10 with the optically transmissive side walls by simply locating the optics assemblies 216, 218 next to the bottom edges of the plates 190 so that when the chamber of the vessel is placed between the plates, the optics assemblies 216, 218 directly contact, or are in close proximity to, 15 the side walls.

Fig. 34 shows a partially cut-away, isometric view of the chamber of the vessel inserted between the plates 190A, 190B (the top portion of the vessel is cut away). The 20 vessel preferably has an angled bottom portion (e.g., triangular) formed by the optically transmissive side walls 57A, 57B. Each of the plates 190A, 190B has a correspondingly shaped bottom portion. The bottom portion of the first plate 190A has a first bottom edge 250A and a 25 second bottom edge 2190B. Similarly, the bottom portion of the second plate 190B has a first bottom edge 252A and a second bottom edge 252B. The first and second bottom edges of each plate are preferably angularly offset from each other by the same angle that the side walls 57A, 57B are 30 offset from each other (e.g., 90°). Additionally, the plates 190A, 190B are preferably positioned to receive the chamber of the vessel between them such that the first side wall 57A is positioned substantially adjacent and parallel to each of the first bottom edges 250A, 252A and such that the

second side wall 57B is positioned substantially adjacent and parallel to each of the second bottom edges 2190B, 252B. This arrangement provides for easy optical access to the optically transmissive side walls 57A, 57B and hence to 5 the chamber of the vessel. A gel or fluid may optionally be used to establish or improve optical communication between each optics assembly and the side walls 57A, 57B. The gel or fluid should have a refractive index close to the refractive indexes of the elements that it is coupling.

10

Referring again to Fig. 28, the optics assemblies 216, 218 are preferably arranged to provide a 90° angle between excitation and detection paths. The 90° angle between excitation and detection paths assures that a minimum 15 amount of excitation radiation entering through the first side wall of the chamber exits through the second side wall. Also, the 90° angle permits a maximum amount of emitted radiation to be collected through the second side wall. In the preferred embodiment, the vessel 40 includes a 20 locating tab 58 (see Fig. 22) that fits into a slot formed between the optics assemblies 216, 218 to ensure proper positioning of the vessel 40 for optical detection. For improved detection, the module 147 also preferably includes a light-tight lid (not shown) that is placed over the top 25 of the vessel 40 and made light-tight to the housing 208 after the vessel is inserted between the plates 190.

Although it is presently preferred to locate the optics assemblies 216, 218 next to the bottom edges of the plates 30 190, many other arrangements are possible. For example, optical communication may be established between the optics assemblies 216, 218 and the walls of the vessel 40 via optical fibers, light pipes, wave guides, or similar

devices. One advantage of these devices is that they eliminate the need to locate the optics assemblies 216, 218 physically adjacent to the plates 190. This leaves more room around the plates in which to circulate cooling air or 5 refrigerant, so that cooling may be improved.

The heat-exchanging module 147 also includes a PC board 226 for holding the electronic components of the module and an edge connector 224 for connecting the module 147 to the 10 instrument 140 (Fig. 10). The heating elements and temperature sensors on the plates 190, as well as the optical boards 220, 222, are connected to the PC board 226 by flex cables (not shown in Fig. 28 for clarity of illustration). The module 147 may also include a grounding 15 trace 228 for shielding the optical detection circuit. The module 147 may optionally include an indicator, such as an LED 214, for indicating to a user the current status of the module such as "heating," "cooling," "finished," or "fault".

20

The housing 208 may be molded from a rigid, high-performance plastic, or other conventional material. The primary functions of the housing 208 are to provide a frame for holding the plates 190, optics assemblies 216, 218, fan 25 212, and PC board 226. The housing 208 also preferably provides flow channels and ports for directing cooling air from the fan 212 across the surfaces of the plates 190 and out of the housing. In the preferred embodiment, the housing 208 comprises complementary pieces (only one piece 30 shown in the schematic side view of Fig. 28) that fit together to enclose the components of the module 147 between them.

Referring again to Fig. 23, the plates 190A, 190B may be made of various thermally conductive materials including ceramics or metals. Suitable ceramic materials include aluminum nitride, aluminum oxide, beryllium oxide, and 5 silicon nitride. Other materials from which the plates may be made include, e.g., gallium arsenide, silicon, silicon nitride, silicon dioxide, quartz, glass, diamond, polyacrylics, polyamides, polycarbonates, polyesters, polyimides, vinyl polymers, and halogenated vinyl polymers, 10 such as polytetrafluoroethylenes. Other possible plate materials include chrome/aluminum, superalloys, zircaloy, aluminum, steel, gold, silver, copper, tungsten, molybdenum, tantalum, brass, sapphire, or any of the other numerous ceramic, metal, or polymeric materials available 15 in the art.

Ceramic plates are presently preferred because their inside surfaces may be conveniently machined to very high smoothness for high wear resistance, high chemical 20 resistance, and good thermal contact to the flexible walls of the reaction vessel. Ceramic plates can also be made very thin, preferably between about 0.6 and 1.3 mm, for low thermal mass to provide for extremely rapid temperature changes. A plate made from ceramic is also both a good 25 thermal conductor and an electrical insulator, so that the temperature of the plate may be well controlled using a resistive heating element coupled to the plate.

Various thermal elements may be employed to heat and/or 30 cool the plates 190A, 190B and thus control the temperature of the reaction mixture in the chamber 42. In general, suitable heating elements for heating the plate include conductive heaters, convection heaters, or radiation heaters. Examples of conductive heaters include resistive

or inductive heating elements coupled to the plates, e.g., resistors or thermoelectric devices. Suitable convection heaters include forced air heaters or fluid heat-exchangers for flowing fluids past the plates. Suitable radiation heaters include infrared or microwave heaters. Similarly, various cooling elements may be used to cool the plates. For example, various convection cooling elements may be employed such as a fan, peltier device, refrigeration device, or jet nozzle for flowing cooling fluids past the surfaces of the plates. Alternatively, various conductive cooling elements may be used, such as a heat sink, e.g. a cooled metal block, in direct contact with the plates.

Referring to Fig. 24, each plate 190 preferably has a resistive heating element 206 disposed on its outer surface. The resistive heating element 206 is preferably a thick or thin film and may be directly screen printed onto each plate 190, particularly plates comprising a ceramic material, such as aluminum nitride or aluminum oxide. Screen-printing provides high reliability and low cross-section for efficient transfer of heat into the reaction chamber. Thick or thin film resistors of varying geometric patterns may be deposited on the outer surfaces of the plates to provide more uniform heating, for example by having denser resistors at the extremities and thinner resistors in the middle. Although it is presently preferred to deposit a heating element on the outer surface of each plate, a heating element may alternatively be baked inside of each plate, particularly if the plates are ceramic. The heating element 206 may comprise metals, tungsten, polysilicon, or other materials that heat when a voltage difference is applied across the material. The heating element 206 has two ends which are connected to respective contacts 204 which are in turn connected to a voltage

source (not shown in Fig. 24) to cause a current to flow through the heating element. Each plate 190 also preferably includes a temperature sensor 192, such as a thermocouple, thermistor, or RTD, which is connected by two traces 202 to 5 respective ones of the contacts 204. The temperature sensor 192 is used to monitor the temperature of the plate 190 in a controlled feedback loop.

The plates have a low thermal mass to enable rapid heating 10 and cooling of the plates. In particular, it is presently preferred that each of the plates has a thermal mass less than about 5 J/ $^{\circ}$ C, more preferably less than 3 J/ $^{\circ}$ C, and most preferably less than 1 J/ $^{\circ}$ C. As used herein, the term thermal mass of a plate is defined as the specific heat of 15 the plate multiplied by the mass of the plate. In addition, each plate should be large enough to cover a respective major wall of the reaction chamber. In the presently preferred embodiment, for example, each of the plates has a width X in the range of 2 to 22 mm, a length Y 20 in the range of 2 to 22 mm, and a thickness in the range of 0.5 to 5 mm. The width X and length Y of each plate is selected to be slightly larger than the width and length of the reaction chamber. Moreover, each plate preferably has an angled bottom portion matching the geometry of the 25 bottom portion of the reaction chamber, as previously described with reference to Fig. 34. Also in the preferred embodiment, each of the plates is made of aluminum nitride having a specific heat of about 0.75 J/g  $^{\circ}$ C. The mass of each plate is preferably in the range of 0.005 to 5.0 g so 30 that each plate has a thermal mass in the range of .00375 to 3.75 J/ $^{\circ}$ C.

The opposing plates 190 are positioned to receive the chamber of the vessel 40 between them such that the flexible major walls of the chamber contact and conform to the inner surfaces of the plates. It is presently preferred 5 that the plates 190 be held in an opposing relationship to each other using, e.g., brackets, supports, or retainers. Alternatively, the plates 190 may be spring-biased towards each other as described in International Publication Number WO 98/38487, the disclosure of which is incorporated by 10 reference herein. In another embodiment of the invention, one of the plates is held in a fixed position, and the second plate is spring-biased towards the first plate. If one or more springs are used to bias the plates towards each other, the springs should be sufficiently stiff to 15 ensure that the plates are pressed against the flexible walls of the vessel with sufficient force to cause the walls to conform to the inner surfaces of the plates.

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Figs. 29-30 illustrate a preferred support structure 209 20 for holding the plates 190A, 190B in an opposing relationship to each other. Fig. 29 shows an exploded view of the structure, and Fig. 30 shows an assembled view of the structure. For clarity of illustration, the support structure 209 and plates 190A, 190B are shown upside down 25 relative to their normal orientation in the heat-exchanging module of Fig. 28. Referring to Fig. 29, the support structure 209 includes a mounting plate 210 having the slot 148 formed therein. The slot 148 is sufficiently large to enable the chamber of the vessel to be inserted through it. 30 Spacing posts 230A, 230B extend from the mounting plate 210 on opposite sides of the slot 148. Spacing post 230A has indentations 232 formed on opposite sides thereof (only one side visible in the isometric view of Fig. 29), and spacing post 230B has indentations 234 formed on opposite sides

thereof (only one side visible in the isometric view of Fig. 29). The indentations 232, 234 in the spacing posts are for receiving the edges of the plates 190A, 190B. To assemble the structure, the plates 190A, 190B are placed 5 against opposite sides of the spacing posts 230A, 230B such that the edges of the plates are positioned in the indentations 232, 234. The edges of the plates are then held in the indentations using a suitable retention means. In the preferred embodiment, the plates are retained by 10 retention clips 236A, 236B. Alternatively, the plates 190A, 190B may be retained by adhesive bonds, screws, bolts, clamps, or any other suitable means.

The mounting plate 210 and spacing posts 230A, 230B are 15 preferably integrally formed as a single molded piece of plastic. The plastic should be a high temperature plastic, such as polyetherimide, which will not deform or melt when the plates 190A, 190B are heated. The retention clips 230A, 230B are preferably stainless steel. The mounting plate 210 20 may optionally include indentations 240A, 240B for receiving flex cables 238A, 238B, respectively, that connect the heating elements and temperature sensors disposed on the plates 190A, 190B to the PC board 226 of the heat-exchanging module 147 (Fig. 28). The portion of 25 the flex cables 238A adjacent the plate 190A is held in the indentation 240A by a piece of tape 242A, and the portion of the flex cables 238B adjacent the plate 190B is held in the indentation 240B by a piece of tape 242B.

30 Fig. 31 is an isometric view of the assembled support structure 209. The mounting plate 210 preferably includes tabs 246 extending from opposite sides thereof for securing the structure 209 to the housing of the heat-exchanging module. Referring again to Fig. 28, the housing 208

preferably includes slots for receiving the tabs to hold the mounting plate 210 securely in place. Alternatively, the mounting plate 210 may be attached to the housing 208 using, e.g., adhesive bonding, screws, bolts, clamps, or 5 any other conventional means of attachment.

Referring again to Fig. 29, the support structure 209 preferably holds the plates 190A, 190B so that their inner surfaces are angled very slightly towards each other. In 10 the preferred embodiment, each of the spacing posts 230A, 230B has a wall 244 that is slightly tapered so that when the plates 190A, 190B are pressed against opposite sides of the wall, the inner surfaces of the plates are angled slightly towards each other. As best shown in Fig. 23, the 15 inner surfaces of the plates 190A, 190B angle towards each other to form a slightly V-shaped slot into which the chamber 42 is inserted. The amount by which the inner surfaces are angled towards each other is very slight, preferably about 1° from parallel. The surfaces are angled 20 towards each other so that, prior to the insertion of the chamber 42 between the plates 190A, 190B, the bottoms of the plates are slightly closer to each other than the tops. This slight angling of the inner surfaces enables the chamber 42 of the vessel to be inserted between the plates 25 and withdrawn from the plates more easily. Alternatively, the inner surfaces of the plates 190A, 190B could be held parallel to each other, but insertion and removal of the vessel 40 would be more difficult.

30 In addition, the inner surfaces of the plates 190A, 190B are preferably spaced from each other a distance equal to the thickness of the frame 46. In embodiments in which the inner surfaces are angled towards each other, the centers of the inner surfaces are preferably spaced a distance

equal to the thickness of the frame 46 and the bottoms of the plates are initially spaced a distance that is slightly less than the thickness of the frame 46. When the chamber 42 is inserted between the plates 190A, 190B, the rigid 5 frame 46 forces the bottom portions of the plates apart so that the chamber 42 is firmly sandwiched between the plates. The distance that the plates 190A, 190B are wedged apart by the frame 46 is usually very small, e.g., about 0.035 mm if the thickness of the frame is 1 mm and the 10 inner surfaces are angled towards each other by 1°.

Referring again to Fig. 30, the retention clips 236A, 236B should be sufficiently flexible to accommodate this slight outward movement of the plates 190A, 190B, yet sufficiently 15 stiff to hold the plates within the recesses in the spacing posts 230A, 230B during insertion and removal of the vessel. The wedging of the vessel between the plates 190A, 190B provides an initial preload against the chamber and ensures that the flexible major walls of the chamber, when 20 pressurized, establish good thermal contact with the inner surfaces of the plates.

Referring again to Fig. 28, to limit the amount that the plates 190 can spread apart due to the pressurization of 25 the vessel 40, stops may be molded into the housings of optics assemblies 216, 218. As shown in Fig. 32, the housing 249 of the optics assembly 218 includes claw-like stops 247A, 247B that extend outwardly from the housing. As shown in Fig. 33, the housing 249 is positioned such that 30 the bottom edges of the plates 190A, 190B are inserted between the stops 247A, 247B. The stops 247A, 247B thus prevent the plates 190A, 190B from spreading farther than a predetermined maximum distance from each other. Although not shown in Fig. 33 for illustrative clarity, the optics

assembly 216 (see Fig. 28) has a housing with corresponding stops for preventing the other halves of the plates from spreading farther than the predetermined maximum distance from each other. Referring again to Fig. 23, the maximum 5 distance that stops permit the inner surfaces of the plates 190A, 190B to be spaced from each other should closely match the thickness of the frame 46. Preferably, the maximum spacing of the inner surfaces of the plates 190A, 190B is slightly larger than the thickness of the frame 46 10 to accommodate tolerance variations in the vessel 40 and plates 190A, 190B. For example, the maximum spacing is preferably about 0.1 to 0.3 mm greater than the thickness of the frame 46.

15 Fig. 35 is a schematic, block diagram of the electronic components of the heat-exchanging module 147. The module includes a connector 224 or flex cable for connection to the main logic board of the instrument. The module also includes heater plates 190A, 190B each having a resistive 20 heating element as described above. The plates 190A, 190B are wired in parallel to receive power input 253 from the instrument. The plates 190A, 190B also include temperature sensors 192A, 192B that output analog temperature signals to an analog-to-digital converter 264. The converter 264 25 converts the analog signals to digital signals and routes them to the microcontroller in the instrument through the connector 224.

The heat-exchanging module also includes a cooling system, 30 such as a fan 212, for cooling the plates 190A, 190B and the reaction mixture contained in the vessel inserted between the plates. The fan 212 is activated by switching a power switch 272, which is in turn controlled by a control logic block 270 that receives control signals from the

microcontroller. The module further includes four light sources, such as LEDs 200, for excitation of labeled analytes in the reaction mixture and four detectors 198, preferably photodiodes, for detecting fluorescent emissions from the reaction mixture. The module also includes an adjustable current source 255 for supplying a variable amount of current (e.g., in the range of 0 to 30 mA) to each LED to vary the brightness of the LED. A digital-to-analog converter 260 is connected between the adjustable current source 255 and the microcontroller to permit the microcontroller to adjust the current source digitally.

The adjustable current source 255 is preferably used to ensure that each LED has about the same brightness when activated. Due to manufacturing variances, many LEDs have different brightnesses when provided with the same amount of current. Therefore, it is presently preferred to test the brightness of each LED during manufacture of the heat-exchanging module and to store calibration data in a memory 268 of the module. The calibration data indicates the correct amount of current to provide to each LED. The microcontroller reads the calibration data from the memory 268 and controls the current source 255 accordingly.

25 The module additionally includes a signal conditioning/gain select/offset adjust block 262 comprised of amplifiers, switches, electronic filters, and a digital-to-analog converter. The block 262 adjusts the signals from the detectors 198 to increase gain, offset, and reduce noise.

30 The microcontroller controls block 262 through a digital output register 266. The output register 266 receives data from the microcontroller and outputs control voltages to the block 262. The block 262 outputs the adjusted detector signals to the microcontroller through the analog-to-

digital converter 264 and the connector 224. The module also includes the memory 268, preferably a serial EEPROM, for storing data specific to the module, such as calibration data for the LEDs 200, thermal plates 190A,  
5 190B, and temperature sensors 192A, 192B.

The operation of the cartridge and instrument will now be described. As shown in Fig. 3, a fluid sample to be analyzed is added to the sample chamber 65 through the  
10 sample port 64 and the cap 30 screwed into the port 64 to seal the port shut. Referring to Fig. 10, the cartridge 20 is then placed into the cartridge nest 141 of the instrument 140 for processing. All valves in the cartridge 20 are initially closed when the cartridge is placed into  
15 the instrument 140. When the cartridge is placed in the instrument, the transducer 92 contacts an external surface of the flexible gasket 63 forming the bottom wall of the lysing chamber 86, as shown in Fig. 5.

20 Referring again to Fig. 10, the instrument 140 is preferably computer-controlled to perform the functions described in the following section, e.g., opening and closing valves in the cartridge using valve actuators 142, providing pressure to the cartridge through nozzles 145,  
25 activating the transducer 92, sensing liquid presence or liquid levels using optical sensors 143 and 144, and controlling the heat-exchanging and optical detection module 147. A programmer having ordinary skill in the art will be able to program a microcontroller and/or computer  
30 to perform these functions based upon the following description.

Referring to Fig. 9, liquids are preferably forced to flow through the cartridge using differential pressure. Although

positive pressure is described herein, negative pressure (vacuum) may also be used to control fluid flow in the cartridge. The maximum amount of positive pressure that can be applied is usually limited by the hydrophobic membranes which may reach liquid break-through pressure above 30 psi.

5 The lower limit of pressure is limited by the need to move sample and other fluids through the cartridge sufficiently quickly to meet assay goals. Below 1 psi, for example, sample may not flow efficiently through the filter stack

10 87. Pressure in the range of 6 to 20 psi is generally adequate. The sample flow rate through the cartridge is preferably in the range of 10 to 30 ml/minute. The wash flow rate may be slower, e.g. 6 to 18 ml/minute so that the wash effectively washes the lysing chamber 86.

15 A specific protocol will now be described with reference to Fig. 9 to illustrate the operation of the cartridge. It is to be understood that this is merely an example of one possible protocol and is not intended to limit the scope of

20 the invention. To begin, the cartridge is preferably primed with wash solution from the wash chamber 66 before the fluid sample is forced to flow from the sample chamber 65. To prime the cartridge, valves 111 and 115 are opened and a pressure of 10 psi is applied to the chamber 66

25 through the pressure port 116 for about two seconds. A small portion of the wash solution flows through the channels 117 and 106, through the lysing chamber 86, through the channels 109 and 110, into the U-shaped channel 122, and all the way to the hydrophobic membrane below the

30 pressure port 128.

Following priming, valve 115 and pressure port 116 are closed and valves 107 and 114 are opened. At the same time, a pressure of 20 psi is applied to the sample chamber

65 through the pressure port 105 for about 15 seconds to force the sample to flow through the channel 106, through the filter stack 87 in the chamber 87, through the channels 110, 111, 112 and into the vented waste chamber 68. As the  
5 sample passes the detection region 136 in the channel 106, the reflective optical sensor 144 (Fig. 13) may be used to determine when the sample chamber 65 has been emptied. As the sample liquid flows through the filter stack 87, target cells or viruses in the sample are captured. When a  
10 predetermined volume of sample reaches the waste chamber 68, some of the liquid spills over into the sensor chamber 120, triggering the next step in the protocol.  
Alternatively, instead of using feedback from optical sensors to trigger events, the steps in a predetermined  
15 protocol may simply be timed, e.g., applying predetermined pressures for predetermined durations of time to move known volumes of fluid at known flow rates.

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The flow-through design of the lysing chamber 86 permits  
20 target cells or viruses from a relatively large sample volume to be concentrated into a much smaller volume for amplification and detection. This is important for the detection of low concentration analyte in the sample, such as nucleic acid. In particular, the ratio of the volume of  
25 the sample forced to flow through the lysing chamber 86 to the volume capacity of the chamber 86 is preferably at least 2:1, and more preferably at least 5:1. The volume of sample forced to flow through the chamber 86 is preferably at least 100  $\mu$ l, and more preferably at least 1 ml. In the  
30 presently preferred embodiment, a sample volume of 5 ml is forced to flow through the lysing chamber 86, and the chamber 86 has a volume capacity of about 0.5 ml, so that the ratio is 10:1. In addition, the lysing chamber 86 may be sonicated (e.g., using an ultrasonic horn coupled to a

wall of the chamber) as the sample is forced to flow through the chamber. Sonicating the chamber 86 helps to prevent clogging of the filter stack 87, providing for more uniform flow through the chamber 86. In particular, the 5 sound waves help keep particulate matter or the beads in the filter stack from clogging one or more filters.

In the next step, valves 111, 114, 115 are opened and a pressure of 20 psi is applied to the wash chamber 66 for 10 about seven seconds to force the wash solution to flow through the channels 117 and 106 into the lysing chamber 86. The washing solution washes away PCR inhibitors and contaminants from the lysing chamber 86 and carries them through the channels 109, 110, and 112 into the waste 15 chamber 68. A variety of suitable wash solutions of varying pH, solvent composition, and ionic strength may be used for this purpose and are well known in the art. For example, a suitable washing reagent is a solution of 80mM potassium acetate, 8.3 mM Tris-HCl, pH 7.5, 40 uM EDTA, and 55% ethanol. The lysing chamber 86 may be sonicated (e.g., 20 using an ultrasonic horn coupled to a wall of the chamber) while the wash solution is forced to flow through the chamber. Sonicating the chamber 86 helps to prevent clogging of the filter stack 87, providing for more uniform 25 flow through the chamber 86 as previously described. In addition, the sound waves may help loosen the material to be washed away. When the incremental volume of wash solution reaches the waste chamber 68, some of the liquid spills over into the sensor chamber 121, triggering the 30 next step in the protocol.

In the next step, valve 115 is closed and valve 119 is opened while a pressure of 15 psi is applied to the reagent chamber 67 through the pressure port 118 for about three

seconds. The pressure forces lysing reagent to flow from the chamber 67 through the channels 117, 106 into the lysing chamber 86, and into the channel 110. The chamber 86 is thus filled with liquid. Suitable lysing reagents 5 include, e.g., solutions containing a chaotropic salt, such as guanidine HCl, guanidine thiocyanate, guanidine isothiocyanate, sodium iodide, urea, sodium perchlorate, and potassium bromide. In the presently preferred embodiment, a lysing reagent that is not inhibitory to PCR 10 is used. The lysing reagent comprises 10 mM tris, 5% tween-20, 1 mM tris (2-carboxyethyl phosphine hydrochloride), 0.1 mM Ethylene Glycol-bis (b-amino-ethyl ether)- N,N,N', N' - tetracetic acid. After the lysing chamber 86 is filled with lysing reagent, the valves 111, 15 114 are closed. Valve 119 remains open and a pressure of 20 psi is applied to pressure port 118. The static pressure in the lysis chamber 86 is therefore increased to 20 psi in preparation for the lysis of the cells or viruses trapped 20 in the filter stack 87.

Referring again to Fig. 5, the pressurization of the lysing chamber 86 is important because it ensures effective coupling between the transducer 92 and the flexible wall 63 of the lysing chamber 86. To disrupt the cells or viruses 25 in the chamber 86, the transducer 92 is activated (i.e., set into vibratory motion). The flexible wall 63 of the lysing chamber 86 transfers the vibratory motion of the transducer 92 to the liquid in the chamber 86 by allowing slight deflections without creating high stresses in the 30 wall. The wall 63 may be formed by the elastomeric membrane as previously described. Alternatively, the wall may be a film or sheet of polymeric material (e.g., a polypropylene film) preferably having a thickness in the range of 0.025 to 0.1 mm. The transducer 92 is preferably an ultrasonic

horn for sonicating the chamber 86. The chamber 86 is preferably sonicated for 10 to 40 seconds at a frequency in the range of 20 to 60 kHz. In the exemplary protocol, the chamber is sonicated for 15 seconds at a frequency of 47 5 kHz. The amplitude of the horn tip is preferably in the range of 20 to 25  $\mu\text{m}$  (measured peak to peak).

As the tip of the transducer 92 vibrates, it repeatedly impacts the flexible wall 63. On its forward stroke (in the 10 upward direction in Fig. 6), the tip of the transducer 92 pushes the wall 63 and creates a pressure pulse or pressure wave in the chamber 86. On its retreating stroke (downward in Fig. 5), the tip of the transducer 92 usually separates from the flexible wall 63 because the flexible wall 63 15 cannot move at the same frequency as the transducer. On its next forward stroke, the tip of the transducer 92 once again impacts the wall 63 in a head-on collision as the tip and wall speed towards each other. Because the transducer 92 and the wall 63 separate as the transducer 92 vibrates, 20 the effective forward stroke of the transducer is less than its peak-to-peak amplitude. The effective forward stroke determines the level of sonication in the chamber 86. It is therefore important to increase the static pressure in the lysing chamber 86 so that when the tip of the transducer 92 25 retreats, the flexible wall 63 is forced outwardly to meet the tip on its return stroke. The static pressure in the chamber 86 should be sufficient to ensure that the effective forward stroke of the transducer 92 generates pressure pulses or pressure waves in the chamber 86. It is 30 presently preferred to increase the static pressure in the chamber 86 to at least 5 psi above the ambient pressure external to the cartridge, and more preferably to a pressure in the range of 15 to 25 psi above the ambient pressure.

On each forward stroke, the transducer 92 imparts a velocity to the liquid in the chamber 86, thus creating a pressure wave that quickly sweeps across the chamber 86.

- 5 The beads in the filter stack 87 (Fig. 6) are agitated by the pressure waves in the chamber 86. The pressure waves propel the beads into violent motion in the chamber 86, and the beads mechanically rupture the cells or viruses to release the material (e.g., nucleic acid) therefrom. It
- 10 should be noted that some types of cells, such as blood cells, are relatively weak and may be disrupted using only pressure waves (e.g., ultrasonic waves) without the use of beads. Other types of cells (particularly spores) have highly resistant cell walls and beads are generally
- 15 required for effective lysis.

Referring again to Fig. 9, following disruption of the cells or viruses, valves 111, 124 are opened and a pressure of 12 psi is delivered for about 4 seconds to the reagent chamber 67 through the pressure port 118. The pressure forces the lysis reagent to elute the nucleic acid from the filter stack 87 and to flow with the nucleic acid into the neutralization chamber 70. The lysing chamber 86 may be sonicated (e.g., using an ultrasonic horn coupled to a wall 20 of the chamber) while the eluting the nucleic acid.

Sonicating the chamber 86 may help prevent clogging of the filter stack 87, as previously described. The chamber 420 is partially filled (e.g., half-filled) with neutralizer, such as detergent, for neutralizing the lysing reagent. If 25 a lysing reagent non-inhibitory to PCR is used, the neutralizer is optional.

In the next step, the valve 124 is closed to hold the lysing reagent, analyte, and neutralizer in the chamber 70.

The valve 114 is opened and a pressure of 15 psi is applied for about three seconds through the pressure port 128 to force any liquid in the U-shaped channel 122 to flow into the waste chamber 68. Next, valves 124 and 126 are opened 5 and a pressure of 15 psi is applied for about five seconds through the pressure port 123 on top of the neutralizer chamber 70. The pressure forces the neutralized lysing reagent and nucleic acid in the chamber 70 to flow into the channel 122 and into the master mix chamber 71. The valve 10 126 to the master mix chamber 71 is then closed. The master mix chamber contains PCR reagents and fluorescent probes that mix with the neutralized lysing reagent and nucleic acid to form a reaction mixture.

15 In the next step, the channel 122 is cleared by opening valve 114 to waste chamber 68 and applying a pressure of 15 psi for about one second to pressure port 128. In the next step, the reaction mixture formed in the master mix chamber 71 is moved into the reaction vessel 40 as follows. Valves 20 126, 127, and 133 are opened and a pressure of 15 psi is applied for about six seconds to the pressure port 125 on top of the master mix chamber 71 to force the reaction mixture to flow through the channel 122, valve 127, and channel 80 into the reaction vessel 40 through the port 41.

25 The reaction mixture fills the chamber 42 of the vessel, displacing air in the chamber which exits through the outlet channel 52. The air escaping through the outlet channel 52 travels in channel 81 past sensor region 130 and into channel 131. From channel 131, the air flows into 30 channel 132, through valve 133, channel 134, and exits the cartridge through the vent 36. When a volume of reaction mixture sufficient to fill the chamber 42 has flowed into the vessel, excess reaction mixture exits the vessel through the outlet channel 52. The excess reaction mixture 35 flows into channel 81 and is optically detected in the sensor region 130. When the reaction mixture is detected, valve 133 is closed while pressure from the pressure port 125 is applied to pressurize the reaction chamber 42.

Referring again to Fig. 23, the pressurization of the chamber 42 expands the flexible major walls 48 of the vessel. In particular the pressure forces the major walls 5 48 to contact and conform to the inner surfaces of the plates 190A, 190B. This ensures optimal thermal conductance between the plates 190A, 190B and the reaction mixture in the chamber 42. It is presently preferred to pressurize the chamber 42 to a pressure in the range of 2 to 30 psi above 10 ambient pressure. This range is presently preferred because 2 psi is generally enough pressure to ensure conformity between the walls 48 and the surfaces of the plates 190A, 190B, while pressures above 30 psi may cause bursting of the walls 48, deformation of the frame 46 or 15 plates 190A, 190B, or bursting of the hydrophobic membranes in the cartridge. More preferably, the chamber 42 is pressurized to a pressure in the range of 8 to 15 psi above ambient pressure. This range is more preferred because it 20 is safely within the practical limits described above. When the chamber 42 is pressurized, the reaction mixture in the vessel 40 is thermally processed and optically interrogated to determine the presence or absence of a target analyte in the mixture.

25 Referring again to Fig. 35, the reaction mixture is thermally processed between the plates 190A, 190B using standard proportional-integral-derivative (PID) control using target temperatures and feedback signals from the temperature sensors 192A, 192B. Proportioning may be 30 accomplished either by varying the ratio of "on" time to "off" time, or, preferably with proportional analog outputs which decrease the average power being supplied either to the heating elements on the plates 190A, 190B or to the fan 212 as the actual temperature of the plates 190A, 190B

approaches the desired set point temperature. PID control combines the proportional mode with an automatic reset function (integrating the deviation signal with respect to time) and rate action (summing the integral and deviation 5 signal to shift the proportional band). Standard PID control is well known in the art and need not be described further herein. Alternatively, the reaction mixture may be thermally processed using a modified version of PID control described in International Publication Number WO 99/48608 10 (Application Number PCT/US99/06628) the disclosure of which is incorporated by reference herein.

As the reaction mixture is thermally cycled between the heater plates 190A, 190B to amplify one or more target 15 nucleic acid sequences in the mixture, the mixture is optically interrogated, preferably at the lowest temperature point in each cycle. Optical interrogation is accomplished by sequentially activating each of the LEDs 200 to excite different fluorescently-labeled analytes in 200 to excite different fluorescently-labeled analytes in 20 the mixture and by detecting light emitted (fluorescent output) from the chamber 42 using detectors the 198. Referring again to Fig. 22, excitation beams are preferably transmitted to the chamber 42 through the optically transmissive side wall 57A, while fluorescent emission is 25 detected through the side wall 57B.

One advantage of the cartridge of the present invention is that it allows the intracellular material from a relatively large volume of fluid sample, e.g. several milliliters or 30 more, to be separated from the sample and concentrated into a much smaller volume of reaction fluid, e.g., 100 µL or less. The cartridge permits extraordinary concentration factors by efficiently extracting material from milliliter quantities of fluid sample. In particular, the sample 35 chamber 65 preferably has a volume capacity in the range of

100  $\mu$ l to 12 ml. More preferably, the sample chamber 65 has a volume capacity of at least 1 ml. The lower limit of 1 ml is preferred because at least 1 ml of sample should be analyzed to detect low concentration analytes such as

5 nucleic acid. The upper limit of 12 ml is preferred because a sample volume greater than 12 ml would require a much larger cartridge and likely clog the filter stack. In the presently preferred embodiment, the sample chamber has a volume capacity of 5.5 ml for holding 5 ml of sample.

10 The wash chamber 66 has a volume capacity proportional to the volume of the lysing chamber 86. In particular, the wash chamber 66 preferably holds a volume of wash that is at least one to two times the volume of the lysing chamber

15 86 to ensure that there is enough wash solution to wash out PCR inhibitors and debris from the chamber 86. In the presently preferred embodiment, the volume of the lysing chamber 86 is about 0.5 ml and the volume of the wash chamber 66 is 2.5 ml for holding 2 ml of wash solution.

20 The lysing chamber volume of 0.5 ml is a compromise between a size large enough to avoid clogging of the filter stack 87 and a size small enough to concentrate analyte into a small volume for improved amplification and detection.

25 The reagent chamber 67 preferably holds a volume of lysing reagent that is at least one to two times the volume of the lysing chamber 86 so that there is sufficient lysing reagent to pressurize the chamber and to elute nucleic acid from the chamber. In the presently preferred embodiment,

30 the chamber 67 has a volume capacity of 1.5 ml for holding about 1 to 1.5 ml of lysing reagent. The waste chamber 68 has a volume capacity sufficient to hold the sample, wash solution, and unused lysing reagent. The waste chamber 68 is sized at 9.5 ml volume capacity in the preferred

35 embodiment.

The size of the neutralization chamber 70 is dependent upon the volume of the lysing chamber 86 since the neutralizer

in the chamber 70 neutralizes the volume of lysing reagent that fills the lysing chamber 86. It is currently preferred that the lysing chamber have a volume if 0.5 ml, so the chamber 70 has a volume capacity of 1.0 ml for holding about 0.5 ml of neutralizer that is mixed with 0.5 ml of the lysing reagent and eluted analyte. The volume capacity of the master mix chamber 71 should be sufficient to produce a reaction mixture to fill the vessel 40 and the channels 122, 127 leading to the vessel. In the presently preferred embodiment, the master mix chamber has a volume capacity of 200  $\mu$ l for holding an initial load of 100  $\mu$ l of master mix to which is added 100  $\mu$ l of neutralized lysing reagent and eluted analyte to form the reaction mixture.

15 The flow channels in the cartridge are generally D-shaped in cross section (with the gasket 63 forming the flat side of the channel) and preferably have a width or diameter in the range of 1/64 to 1/8 of an inch (0.4 to 3.2 mm), and more preferably a width of 1/32 to 1/16 of an inch (0.8 to 20 1.6 mm). These ranges are presently preferred to avoid having channels too narrow (which creates flow restriction) and to avoid having channels too wide (which yields unused volumes of liquid sitting in the flow path).

25 Many modifications to the structure and operation of the cartridge and instrument are possible in alternative embodiments. For example, although amplification by PCR is presently preferred, the cartridge and instrument may be used to amplify nucleic acid sequences using any 30 amplification method, including both thermal cycling amplification methods and isothermal amplification methods. Suitable thermal cycling methods include, but are not limited to, the Polymerase Chain Reaction (PCR; U.S Pat. Nos. 4,683,202, 4,683,195 and 4,965,188); Reverse 35 Transcriptase PCR (RT-PCR); DNA Ligase Chain Reaction (LCR; International Patent Application No. WO 89/09835); and

transcription-based amplification (D. Y. Kwoh et al. 1989, Proc. Natl. Acad. Sci. USA 86, 1173-1177). Suitable isothermal amplification methods useful in the practice of the present invention include, but are not limited to,

5 Rolling Circle Amplification; Strand Displacement Amplification (SDA; Walker et al. 1992, Proc. Nati. Acad. Sci. USA 89, 392-396); Q-beta. replicase (Lizardi et al. 1988, Bio/Technology 6, 1197-1202); Nucleic Acid-Based Sequence Amplification (NASBA; R. Sooknanan and L. Malek 10 1995, Bio/Technology 13, 563-65); and Self-Sustained Sequence Replication (3SR; Guatelli et al. 1990, Proc. Nati. Acad. Sci. USA 87, 1874-1878).

Moreover, the cartridge and instrument may be used to

15 conduct chemical reactions other than nucleic acid amplification. Further, although fluorescence excitation and emission detection is preferred, optical detection methods such as those used in direct absorption and/or transmission with on-axis geometries may also be used to

20 detect analyte in the cartridge. Another possible detection method is time decay fluorescence. Additionally, the cartridge is not limited to detection based upon fluorescent labels. For example, detection may be based upon phosphorescent labels, chemiluminescent labels, or

25 electrochemiluminescent labels.

A fluid sample may be introduced into the cartridge by a variety of means, manual or automated. For manual addition, a measured volume of material may be placed into

30 a receiving area of the cartridge through an input port and a cap is then placed over the port. Alternatively, a greater amount of sample material than required for the analysis can be added to the cartridge and mechanisms within the cartridge can effect the precise measuring and

35 aliquoting of the sample needed for the specified protocol.

It may be desirable to place certain samples, such as tissue biopsy material, soil, feces, exudates, and other complex material into another device or accessory and then place the secondary device or accessory into the cartridge.

5 For example, a piece of tissue may be placed into the lumen of a secondary device that serves as the cap to the input port of the cartridge. When the cap is pressed into the port, the tissue is forced through a mesh that slices or otherwise divides the tissue.

10

For automated sample introduction, additional design features of the cartridge are employed and, in many cases, impart specimen accession functionality directly into the cartridge. With certain samples, such as those presenting a 15 risk of hazard to the operator or the environment, such as human retrovirus pathogens, the transfer of the sample to the cartridge may pose a risk. Thus, in one embodiment, a syringe may be integrated into a device to provide a means for moving external fluidic samples directly into the 20 cartridge. Alternatively, a venous puncture needle and an evacuated blood tube can be attached to the cartridge forming an assembly that can be used to acquire a sample of blood. After collection, the tube and needle are removed and discarded, and the cartridge is then placed in an 25 instrument to effect processing. The advantage of such an approach is that the operator or the environment is not exposed to pathogens.

The input port can be designed with a consideration of 30 appropriate human factors as a function of the nature of the intended specimen. For example, respiratory specimens may be acquired from the lower respiratory tract as expectorants from coughing, or as swab or brush samples from the back of the throat or the nares. In the former 35 case, the input port can be designed to allow the patient to cough directly into the cartridge or to otherwise facilitate spitting of the expectorated sample into the cartridge. For brush or swab specimens, the specimen is

placed into the input port where features of the port and closure facilitate the breaking off and retaining of the end of the swab or brush in the cartridge receiving area.

- 5 In another embodiment, the cartridge includes input and output tubes that may be positioned in a sample pool of very large volume, such as a flowing stream of water, so that the sample material flows through the cartridge. Alternatively, a hydrophilic wicking material can serve as
- 10 an interactive region so that the entire cartridge can be immersed directly into the specimen, and a sufficient amount of specimen is absorbed into the wicking material. The cartridge is then removed, and can be transported to the laboratory or analyzed directly using a portable
- 15 instrument. In another embodiment, tubing can be utilized so that one end of the tube is in direct communication with the cartridge to provide a fluidic interface with at least one interactive region and the other end is accessible to the external environment to serve as a receiver for sample.
- 20 The tube can then be placed into a specimen and serve as a sipper. The cartridge itself may also serve as the actual specimen collection device, thereby reducing handling and inconvenience. In the case of specimens involved in legal disputes or criminal investigations, the direct accessing
- 25 of the test material into the fluidic cartridge is advantageous because the chain of custody is conveniently and reliably preserved.

Referring again to Fig. 9, reagents may be exogenously introduced into the cartridge before use, e.g., through sealable openings in the reagent chamber 67, neutralizer chamber 70, and master mix chamber 71. Alternatively, the reagents may be placed in the cartridge during manufacture, e.g., as aqueous solutions or dried reagents requiring reconstitution. The particular format is selected based on a variety of parameters, including whether the interaction is solution-phase or solid-phase, the inherent thermal stability of the reagent, speed of reconstitution, and

reaction kinetics. Reagents containing compounds that are thermally unstable when in solution can be stabilized by drying using techniques such as lyophilization. Additives, such as simple alcohol sugars, methylcelluloses, and 5 bulking proteins may be added to the reagent before drying to increase stability or reconstitutability.

Referring again to Fig. 21, the reaction vessel 40 does not require two flexible sheets forming opposing major walls 48 of the reaction chamber 42. For example, in one alternative embodiment, the vessel 40 has only one flexible sheet forming a major wall of the chamber. The rigid frame 46 defines the other major wall of the chamber, as well as the side walls of the chamber. In this embodiment, the major 15 wall formed by the frame 46 should have a minimum thickness of about 0.05 inches (1.25 mm) which is typically the practical minimum thickness for injection molding, while the flexible sheet may be as thin as 0.0005 inches (0.0125 mm). The advantage to this embodiment is that the 20 manufacturing of the reaction vessel 40 is simplified, and hence less expensive, since only one flexible sheet need be attached to the frame 46. The disadvantage is that the heating and cooling rates of the reaction mixture are likely to be slower since the major wall formed by the 25 frame 46 will probably not permit as high a rate of heat transfer as the thin, flexible sheet.

Referring to Fig. 28, the heat-exchanging module 147 only requires one thermal surface for contacting a flexible wall 30 of the reaction vessel 40 and one thermal element for heating and/or cooling the thermal surface. The advantage to using one thermal surface and one thermal element is that the apparatus may be manufactured less expensively. The disadvantage is that the heating and cooling rates are 35 likely to be about twice as slow. Further, although it is

presently preferred that the thermal surfaces be formed by the thermally conductive plates 190, each thermal surface may be provided by any rigid structure having a contact area for contacting a wall of the vessel 40. The thermal 5 surface preferably comprises a material having a high thermal conductivity, such as ceramic or metal. Moreover, the thermal surface may comprise the surface of the thermal element itself. For example, the thermal surface may be the surface of a thermoelectric device that contacts the wall 10 to heat and/or cool the chamber.

It is presently preferred to build the transducer into the instrument 140. In another embodiment, however, the transducer may be built into the cartridge. For example, a 15 piezoelectric disk may be built into the cartridge for sonicating the lysing chamber. Alternatively, a speaker or electromagnetic coil device may be built into the cartridge. In these embodiments, the cartridge includes suitable electrical connectors for connecting the 20 transducer to a power supply. In embodiments in which the transducer is built into the cartridge, the transducer should be prevented from contacting the fluid sample directly, e.g., the transducer should be laminated or separated from the sample by a chamber wall. Further, lysis 25 of the cells or viruses may be performed using a heater in place of or in combination with a transducer. The heater may be a resistive heating element that is part of cartridge, or the heater could be built into the instrument that receives the cartridge. In this embodiment, the cells 30 or viruses are disrupted by heating the lysis chamber to a high temperature (e.g., 95°C) to disrupt the cell walls.

Figs. 36-46 show another apparatus 350 for disrupting cells or viruses according to the present invention. Fig. 36 35 shows an isometric view of the apparatus 350, and Fig. 37

shows a cross sectional view of the apparatus 350. As shown in Figs. 36-37, the apparatus 350 includes a cartridge or container 358 having a chamber 367 for holding the cells or viruses. The container includes a flexible wall 440 defining the chamber 367. In this embodiment, the flexible wall 440 is the bottom wall of the chamber 367. The flexible wall 440 is preferably a sheet or film of polymeric material (e.g., a polypropylene film) and the wall 440 preferably has a thickness in the range of 0.025 to 0.1 mm. The apparatus 350 also includes a transducer 314, such as an ultrasonic horn, for contacting an external surface of the flexible wall 440 (i.e., a surface of the wall 440 that is external to the chamber 367). The transducer 314 should be capable of vibratory motion sufficient to create pressure pulses in the chamber 367. Suitable transducers include ultrasonic, piezoelectric, magnetostrictive, or electrostatic transducers. The transducer may also be an electromagnetic device having a wound coil, such as a voice coil motor or a solenoid device.

The apparatus 350 further includes a support structure 352 for holding the container 358 and the transducer 314 against each other such that the transducer 314 contacts the wall 440 of the chamber 367 and for applying a substantially constant force to the container 358 or to the transducer 314 to press together the transducer 314 and the wall 440 of the chamber. The support structure 352 includes a base structure 354 having a stand 356. The transducer 314 is slidably mounted to the base structure 354 by a guide 364. The guide 364 is either integrally formed with the base structure 354 or fixedly attached to the base structure. The support structure 352 also includes a holder 360 attached to the base structure 354 for holding

the container 358. The holder 360 has a U-shaped bottom portion providing access to the flexible wall 440 of the chamber 367. The guide 364 and the holder 360 are arranged to hold the transducer 314 and the container 358,  
5 respectively, such that the external surface of the wall 440 contacts the transducer 314. The support structure 352 also includes a top retainer 362 for the container 358. The retainer 362 is U-shaped to allow access to an exit port 444 formed in the container 358.

10

The support structure 352 further includes an elastic body, such as a spring 366, for applying a force to the transducer 314 to press the transducer 314 against the wall 440. When the transducer 314 is in contact with the wall 440, the force provided by the spring 366 is constant, providing for consistent coupling between the transducer 314 and the wall 440. The spring 366 is positioned between a spring guide 372 and the base of a coupler 368 that supports the bottom of the transducer 314. As shown in Fig. 36, the coupler 370 preferably has a window 370 through which the power cord (not shown) of the transducer 314 may be placed. Bolts or screws 376 hold the spring guide 372 in adjustment grooves 374 formed in the base structure 354. The magnitude of the force provided by the spring 366 may 25 be adjusted by changing the preload on the spring. To adjust the preload on the spring 366, the bolts 376 holding the spring guide 372 are loosened, the guide 372 is moved to a new position, and the bolts 376 are retightened to hold the guide 372 in the new position. Once the preload on 30 the spring 366 is adjusted to provide a suitable coupling force between the transducer 314 and the wall 440, it is desirable to keep the preload constant from one use of the apparatus 350 to the next so that valid comparisons can be made between different samples disrupted by the apparatus.

The magnitude of the force provided by the spring 366 to press together the transducer 314 and the wall 440 is important for achieving a consistent transfer of energy

5 between the transducer 314 and the chamber 367. If the force is too light, the transducer 314 will only be held lightly against the wall 440, leading to poor translation of vibratory movement from the transducer 314 to the wall 440. If the force is too strong, the container 358 or wall

10 440 may be damaged during sonication. An intermediate force results in the most consistent and repeatable transfer of vibratory motion from the transducer 314 to the wall 440. It is presently preferred that the spring 366 provide a force in the range of 1 to 5 lbs., with a force of about 2

15 lbs. being the most preferred.

Fig. 38 shows an exploded view of the container 358, and Fig. 39 shows an assembled view of the container 358. As shown in Figs. 38-39, the container 358 has a body

20 comprising a top piece 448, a middle piece 450, and a bottom piece 452. The middle piece 450 defines an inlet port 442 to the chamber 367, and the top piece 448 defines an outlet port 444 to the chamber. The ports 442, 444 are positioned to permit the continuous flow of a fluid sample

25 through the chamber 367. The flexible wall 440 is held between the middle and bottom pieces 450, 452 using gaskets 453, 454. Alternatively, the flexible wall 440 may simply be heat sealed to the middle piece 450 so that the bottom piece 452 and gaskets 453, 454 may be eliminated.

30

The container 358 also includes a filter stack 446 in the chamber 367 for capturing sample components (e.g., target cells or viruses) as the sample flows through the chamber 367. The filter stack comprises (from bottom to top in

Figs. 38-39) a gasket 456, a first filter 458, a gasket 460, a second filter 464 having a smaller average pore size than the first filter 458, and a gasket 466. The filter stack is held between the top and middle pieces 448, 450 of 5 the container 358. The filter stack also includes beads 462 disposed between the first and second filters 458 and 464. The gasket 460 spaces the first filter 458 from the second filter 464. The gasket 460 should be thick enough to permit the beads to move freely in the space between the filters 10 458, 464. A fluid sample flowing through the chamber 367 first flows through the filter 458 and then through the filter 466. After flowing through the filter stack, the sample flows along flow ribs 468 (Fig. 38) formed in the portion of the top piece 448 that defines the top of the 15 chamber and through the outlet port 444 (Fig. 39).

The filter stack is effective for capturing cells or viruses as a fluid sample flows through the chamber 367 without clogging of the. The first filter 458 (having the 20 largest pore size) filters out coarse material such as salt crystals, cellular debris, hair, tissue, etc. The second filter 464 (having a smaller pore size) captures target cells or viruses in the fluid sample. The average pore size of the first filter 458 is selected to be small enough to 25 filter coarse material from the fluid sample (e.g., salt crystals, cellular debris, hair, tissue) yet large enough to allow the passage of the target cells or viruses. In general, the average pore size of the first filter 458 should be in the range of about 2 to 25  $\mu\text{m}$ , with a 30 presently preferred pore size of about 5  $\mu\text{m}$ . The average pore size of the second filter 464 is selected to be slightly smaller than the average size of the target cells or viruses to be captured (typically in the range of 0.2 to 5  $\mu\text{m}$ ).

35

The beads 462 are useful for disrupting the captured cells or viruses to release the intracellular material (e.g.,

nucleic acid) therefrom. Movement of the beads 462 ruptures the cells or viruses captured on the filter 464. Suitable beads for rupturing cells or viruses include borosilicate glass, lime glass, silica, and polystyrene beads. The beads 5 may be porous or non-porous and preferably have an average diameter in the range of 1 to 200  $\mu\text{m}$ . In the presently preferred embodiment, the beads 462 are polystyrene beads having an average diameter of about 100  $\mu\text{m}$ .

10 The beads 462 may have a binding affinity for target cells or viruses in the fluid sample to facilitate capture of the target cells or viruses. For example, antibodies or certain receptors may be coated onto the surface of the beads 462 to bind target cells in the sample. Moreover, the chamber 15 367 may contain two different types of beads for interacting with target cells or viruses. For example, the chamber may contain a first set of beads coated with antibodies or receptors for binding target cells or viruses and a second set of beads (intermixed with the first set)

20 for rupturing the captured cells or viruses. The beads in the chamber may also have a binding affinity for the intracellular material (e.g., nucleic acid) released from the ruptured cells or viruses. Such beads may be useful for isolating target nucleic acid for subsequent elution and 25 analysis. For example, the chamber 367 may contain silica beads to isolate DNA or cellulose beads with oligo dT to isolate messenger RNA for RT-PCR. The chamber 367 may also contain beads for removing unwanted material (e.g., proteins, peptides) or chemicals (e.g., salts, metal ions, 30 or detergents) from the sample that might inhibit PCR.

To ensure that the air bubbles can escape from the chamber 367, it is desirable to use the container 358 in an orientation in which liquid flows up (relative to gravity)

through the filters 458, 464 and the chamber 367. The upward flow through the chamber 367 aids the flow of air bubbles out of the chamber. Thus, the inlet port 442 for entry of fluids into the chamber 367 should generally be at 5 a lower elevation than the outlet port 444. The volume capacity of the chamber 367 is usually in the range of 50 to 500  $\mu$ l. The volume capacity of the chamber 367 is selected to provide for concentration of analyte separated from a fluid sample without the chamber being so small that 10 the filters 458, 464 become clogged.

The pieces 448, 450, 452 forming the body of the container 358 are preferably molded polymeric parts (e.g., polypropylene, polycarbonate, acrylic, etc.). Although 15 molding is preferred for mass production, it also possible to machine the top, middle, and bottom pieces 448, 450, 452. The pieces 448, 450, 452 may be held together by screws or fasteners. Alternatively, ultrasonic bonding, solvent bonding, or snap fit designs could be used to 20 assemble the container 358. Another method for fabricating the container 358 is to mold the body as a single piece and heat seal the flexible wall 440 and the filters 458, 464 to the body.

25 Fig. 40 shows a fluidic system for use with the apparatus. The system includes a bottle 470 for holding lysis buffer, a bottle 472 containing wash solution, and a sample container 474 for holding a fluid sample. The bottles 470, 472 and sample container 474 are connected via tubing to 30 the valve ports of a syringe pump 476. The inlet port of the container 358 is also connected to the syringe pump 476. The outlet port of the container 358 is connected to the common port of a distribution valve 478. The system also includes a collection tube 480 for receiving 35 intracellular material removed from the sample, a waste container 482 for receiving waste, and a pressure source,

such as a pump 484. The collection tube 480, waste container 482, and pump 484 are connected to respective peripheral ports of the distribution valve 478. A pressure regulator 486 regulates the pressure supplied by the pump  
5 484.

A specific protocol will now be described with reference to Figs. 39-40 to illustrate the operation of the container 358. It is to be understood that this is merely an example 10 of one possible protocol and is not intended to limit the scope of the invention. The syringe pump 476 pumps a fluid sample from the sample container 474 through the container 358 and into the waste container 482. As the fluid sample is forced to flow through the filters in the chamber 367, 15 coarse material is filtered by the filter 458 and target cells or viruses in the sample are captured by the filter 464. The chamber 367 may be sonicated as the sample is forced to flow through the chamber to help prevent clogging of the filters. Next, the syringe pump 476 pumps wash 20 solution from the bottle 472 through the container 358 and into the waste container 482. The washing solution washes away PCR inhibitors and contaminants from the chamber 367.

In the next step, the syringe pump 476 pumps lysis buffer 25 from the bottle 470 into the container 358 so that the chamber 367 is filled with liquid. The lysis buffer should be a medium through which pressure waves can be transmitted. For example, the lysis buffer may comprise deionized water for holding the cells or viruses in 30 suspension or solution. Alternatively, the lysis buffer may include one or more lysing agents to aid in the disruption of the cells or viruses. One of the advantages of the present invention, however, is that harsh lysing agents are not required for successful disruption of the cells or 35 viruses. Next, the distribution valve of the syringe pump

476 is closed upstream of the container 358, and the distribution valve 478 is opened. The pump 484 then pressurized the chamber 367 through the outlet port 444, preferably to about 20 psi above the ambient pressure. The 5 distribution valve 478 downstream of the container 358 is then closed. The static pressure in the chamber 367 is therefore increased to about 20 psi in preparation for the disruption of the cells or viruses trapped on the filter 464.

10

Referring again to Fig. 37, the pressurization of the chamber 367 is important because it ensures effective coupling between the transducer 314 and the flexible wall 440. To disrupt the cells or viruses in the chamber 367, 15 the transducer 314 is activated (i.e., set into vibratory motion). The flexible wall 440 transfers the vibrational motion of the transducer 314 to the liquid in the chamber 367 by allowing slight deflections without creating high stresses in the wall. The transducer 314 is preferably an 20 ultrasonic horn for sonicating the chamber 367. The chamber 367 is preferably sonicated for 10 to 40 seconds at a frequency in the range of 20 to 60 kHz. In the exemplary protocol, the chamber is sonicated for 15 seconds at a frequency of 40 kHz. The amplitude of the horn tip is 25 preferably in the range of 20 to 25  $\mu\text{m}$  (measured peak to peak).

As the tip of the transducer 314 vibrates, it repeatedly impacts the flexible wall 440. On its forward stroke (in 30 the upward direction in Fig. 37), the tip of the transducer 314 pushes the wall 440 and creates a pressure pulse or pressure wave in the chamber 367. On its retreating stroke (downward in Fig. 37), the tip of the transducer 314 usually separates from the flexible wall 440 because the 35 flexible wall 440 cannot move at the same frequency as the

transducer. On its next forward stroke, the tip of the transducer 314 once again impacts the wall 440 in a head-on collision as the tip and wall speed towards each other. Because the transducer 314 and the wall 440 separate as the 5 transducer 314 vibrates, the effective forward stroke of the transducer is less than its peak-to-peak amplitude. The effective forward stroke determines the level of sonication in the chamber 367. It is therefore important to increase the static pressure in the chamber 367 so that when the tip 10 of the transducer 314 retreats, the flexible wall 440 is forced outwardly to meet the tip on its return stroke. The static pressure in the chamber 367 should be sufficient to ensure that the effective forward stroke of the transducer 314 generates the necessary pressure pulses or pressure 15 waves in the chamber to effect cell disruption. It is presently preferred to increase the static pressure in the chamber 367 to at least 5 psi above the ambient pressure, and more preferably to a pressure in the range of 15 to 25 psi above the ambient pressure.

20

On each forward stroke, the transducer 314 imparts a velocity to the liquid in the chamber 367, thus creating a pressure wave that quickly sweeps across the chamber. The beads 462 in the filter stack 446 (Fig. 38) are agitated by 25 the pressure waves in the chamber 367. The pressure waves propel the beads into violent motion, and the beads mechanically rupture the cells or viruses to release the analyte (e.g., nucleic acid) therefrom. Referring again to Fig. 40, following disruption of the cells or viruses, the 30 syringe pump 476 pumps the released intracellular material from the container 358 into the collection tube 480.

Fig. 41 shows another embodiment of the invention in which the container 358 has a solid wall 488 for contacting the

transducer 314. The solid wall 488 differs from the flexible wall 440 previously described with reference to Fig. 37. Whereas the flexible wall is typically a thin film that bends under its own weight and does not hold its shape unless held on its edges, the solid wall 488 holds its shape when unsupported. The advantage of using a solid wall to contact the transducer 314 is that there is no need to pressurize the chamber 367 to ensure effective coupling between the wall 488 and the transducer 314. The solid nature of the wall 488 provides the necessary coupling between the wall and the transducer 314. However, the proper design of the solid wall 488 is necessary so that the wall is not damaged (e.g., melted) by the vibratory movements of the transducer 314.

15

In particular, the solid wall 488 should have a natural frequency that is higher than the vibrating frequency at which the transducer 314 is operated. In addition, the wall 488 should not be so rigid that it cannot transfer the vibratory motion of the transducer to the liquid in the chamber 367. It is preferred that the wall 488 be capable of deflecting at least 20  $\mu\text{m}$  peak to peak when the transducer 314 applies a force in the range of 1 to 5 lbs. to the external surface of the wall 488. To achieve these criteria, the wall 488 is dome-shaped and extends outwardly towards the transducer 314. The advantage to the dome-shaped design of the wall 488 is that the dome shape increases the natural frequency of the wall (compared to a flat wall) without causing the wall to be so stiff that it cannot transfer the vibratory movements of the transducer 314 to the chamber 367.

Fig. 42 shows a cross sectional view of the wall 488. The dome-shaped portion 495 of the wall preferably has a radius of curvature R equal to about 9.5 mm and a diameter D1 of about 10 mm. The total diameter D2 of the wall including the flat outer rim 497 is preferably about 14.3 mm. The thickness T of the wall is preferably in the range of 0.25

to 1 mm. If it is less than 0.25 mm thick the wall is difficult to mold. If the wall has a thickness greater than 1 mm, the wall may be too stiff to deflect properly in response to the vibratory movements of the transducer. In 5 the presently preferred embodiment, the wall 488 has a thickness T of about 0.5 mm. The wall 488 is preferably a molded plastic part. Suitable materials for the wall 488 include Delrin® (acetal resins or polymethylene oxide), polypropylene, or polycarbonate.

10

The interaction of the transducer 314 with the solid wall 488 will now be described with reference to Fig. 41. Prior to activating the transducer, target cells or viruses are captured on the filter 490 by forcing a fluid sample to 15 flow though the chamber 367 (e.g., using the fluidic system previously described with reference to Fig. 40). In addition, the chamber 367 is filled with a liquid (e.g., lysis buffer) as previously described. Unlike the previously described embodiments, however, the chamber 367 20 is not pressurized. Instead, ambient pressure is maintained in the chamber. The transducer 314 is placed in contact with the external surface of the wall 488, preferably using a support structure as previously described with reference to Fig. 37. In particular, a spring preferably pushes the 25 transducer against the wall 488 with a force in the range of 1 to 5 lbs.

To disrupt the cells or viruses in the chamber 367, the transducer 314 is activated (i.e., induced into vibratory 30 motion). As the tip of the transducer 314 vibrates, it deflects the wall 488. On its forward stroke (in the upward direction in Fig. 41), the tip of the transducer 314 pushes the wall 488 and creates a pressure pulse or pressure wave in the chamber 367. On its retreating stroke (downward in 35 Fig. 41), the wall 488 remains in contact with the tip of the transducer 314 because the wall 488 has a natural frequency higher than the vibrating frequency of the transducer. In embodiments in which the transducer is an

ultrasonic horn for sonicating the chamber 367, the chamber 367 is preferably sonicated for 10 to 40 seconds at a frequency in the range of 20 to 40 kHz. In the exemplary protocol, the chamber is sonicated for 15 seconds at a 5 frequency of 40 kHz. The amplitude of the horn tip is preferably in the range of 20 to 25  $\mu\text{m}$  (measured peak to peak), and the natural frequency of the wall 488 is at least 40 kHz.

10 One advantage to using the solid interface wall 488 is that strong pressure drops can be achieved in the chamber 367 as long as the static pressure in the chamber is low. For example, at atmospheric pressure, cavitation (the making and breaking of microscopic bubbles) can occur in the 15 chamber 367. As these bubbles or cavities grow to resonant size, they collapse violently, producing very high local pressure changes. The pressure changes provide a mechanical shock to the cells or viruses, resulting in their disruption. The disruption of the cells or viruses may also 20 be caused by sharp pressure rises resulting from the vibratory movement of the transducer 314. In addition, the disruption of the cells or viruses may be caused by the violent motion of the beads 462 in the chamber 367. The beads are agitated by the dynamic pressure pulses in the 25 chamber and rupture the cells or viruses. In experimental testing, the applicants have found that it is usually necessary to use beads to disrupt certain types of cells (particularly spores) having highly resistant cell walls. Other types of cells, such as blood cells, are easier to 30 disrupt and may often be disrupted without the use of the beads 462.

Although the use of an ultrasonic transducer has been described as a preferred embodiment, it is to be understood

that different types of transducers may be employed in the practice of the present invention. The transducer should be capable of creating pressure pulses or pressure waves in the chamber 367. In addition, the transducer should be  
5 capable of providing high velocity impacts to the liquid in the chamber. Suitable transducers include ultrasonic, piezoelectric, magnetostrictive, or electrostatic transducer. The transducer may also be an electromagnetic device having a wound coil, such as a voice coil motor or a  
10 solenoid device. The vibrating frequency of the transducer may be ultrasonic (i.e., above 20 kHz) or below ultrasonic, e.g., in the range of 60 to 20,000 Hz. The advantage to using higher frequencies is that cell disruption is very rapid and can often be completed in 10 to 20 seconds. The  
15 disadvantage is that ultrasonic transducers are often more expensive than a simple mechanical vibrator, e.g., a speaker or electromagnetic coil device. In one alternative embodiment, for example, the solid wall 488 is used in combination with a speaker or electromagnetic coil device  
20 that vibrates at an operating frequency in the range of 5 to 10 kHz.

Figs. 43A-43B illustrate another solid wall 500 for contacting a transducer according to the present invention.  
25 As shown in Fig. 43A, one side of the wall 500 has a central portion 502 and a plurality of stiffening ribs 504 extending radially from the central portion 502. The wall also has recesses 506 formed between the ribs 504. As shown in Fig. 43B, the other side of the wall 500 has a flat  
30 surface 508. Fig. 44 shows a partially-cut away isometric view of the container 358 with the wall 500. The wall 500 is preferably positioned so that the side of the wall having the flat surface is internal to the chamber 367 and such that the side of the wall having the ribs 504 is

external to the chamber. The ribs 504 are advantageous because they increase the natural frequency of the wall without causing the wall to be so stiff that it cannot transfer the vibratory movements of the transducer to the 5 chamber 367.

Fig. 45 shows a bottom plan view of the container 358 having the wall 500. The central portion 502 provides the external surface of the wall 500 for contacting a 10 transducer. The interaction of the wall 500 with the transducer is analogous to the interaction of the wall 488 with the transducer previously described with reference to Fig. 41. In particular, the wall 500 remains in contact with the tip of the transducer because the wall 500 has a 15 natural frequency higher than the vibrating frequency of the transducer. Consequently, pressurization is not required, and cavitation may be achieved. The solid walls 488, 500 described with reference to Figs. 41-45 may be used in the container 358 or the walls 488, 500 may be used 20 in a fully integrated cartridge, such as the cartridge shown in Fig. 1.

Although the above description contains many specificities, these should not be construed as limitations on the scope 25 of the invention, but merely as illustrations of some of the presently preferred embodiments. Many possible variations and modifications to the invention will be apparent to one skilled in the art upon consideration of this disclosure.

30

Therefore, the scope of the invention should be determined by the following claims and their legal equivalents.

**CLAIMS**

What is claimed is:

1. A device for separating a desired analyte from a fluid sample, the device comprising a cartridge having:
  - 5 a) a sample flow path;
  - b) a lysing chamber in the sample flow path, wherein the lysing chamber contains at least one filter for capturing cells or viruses from the sample as the sample flows through the lysing chamber;
  - 10 c) beads disposed in the lysing chamber for rupturing the cells or viruses to release the analyte therefrom;
  - d) a waste chamber in fluid communication with the lysing chamber via the sample flow path for receiving the remaining sample after the sample flows through the lysing chamber;
  - 15 e) an analyte flow path extending from the lysing chamber, wherein the analyte flow path diverges from the sample flow path; and
  - f) at least one flow controller for directing the sample into the waste chamber after the sample flows through the lysing chamber and for directing the analyte separated from the sample into the analyte flow path.
- 25 2. The device of claim 1, wherein the cartridge includes a first filter in the sample flow path for filtering coarse material from the sample and a second filter in the lysing chamber, the second filter having a smaller average pore size than the first filter
- 30 3. The device of claim 2, wherein both the first and second filters are positioned in the lysing chamber, and wherein the beads are disposed between the filters.

4. The device of claim 3, further comprising a third filter in the lysing chamber, wherein the third filter is spaced from the second filter, and the third filter has a smaller average pore size than the second filter.  
5
5. The device of claim 4, wherein the cartridge includes a first set of beads disposed between the first and second filters and a second set of beads disposed  
10 between the second and third filters.
6. The device of claim 5, wherein the average diameter of the beads in the first set differs from the average diameter of the beads in the second set.  
15
7. The device of claim 1, wherein the beads have a binding affinity for the cells to be disrupted.
8. The device of claim 1, wherein the beads have a binding  
20 affinity for the analyte.
9. The device of claim 1, wherein the lysing chamber contains a first set of beads for binding the cells and a second set of beads for rupturing the cells.  
25
10. The device of claim 1, wherein the cartridge further includes:
  - a) a first sensor chamber in fluid communication with the waste chamber for indicating when the waste chamber has received a first predetermined volume of liquid; and  
30
  - b) a first wall having a first spillover rim disposed between the waste chamber and the first sensor chamber for preventing liquid from flowing from the waste chamber into the sensor chamber until the liquid in the waste chamber reaches the  
35 level of the spillover rim.

11. The device of claim 10, wherein the cartridge further includes:
  - a) a second sensor chamber in fluid communication with the waste chamber; and
  - b) a second wall having a second spillover rim disposed between the waste chamber and the second sensor chamber, the second spillover rim being positioned at a different level relative to the bottom of the waste chamber than the first spillover rim.
12. The device of claim 1, further comprising a reaction chamber for receiving the analyte separated from the sample, wherein the reaction chamber is in fluid communication with the lysing chamber via the analyte flow path.
13. The device of claim 1, wherein the cartridge includes at least one wall defining the lysing chamber, and wherein the device further comprises a transducer for contacting an external surface of the wall to generate pressure waves in the lysing chamber.
14. The device of claim 12, wherein the transducer comprises an ultrasonic horn.
15. A method for separating a desired analyte from a fluid sample, the method comprising the steps of:
  - a) introducing the sample into a cartridge having:
    - i) a sample flow path;
    - ii) a first filter in the sample flow path for filtering coarse material from the sample; and
    - iii) a lysing chamber in the sample flow path, wherein the lysing chamber contains a second filter for separating cells or viruses from the sample, the second filter having a smaller average pore size than the first filter;

- b) forcing the sample to flow through the sample flow path, thereby capturing the cells or viruses with the second filter as the sample flows through the chamber, wherein the ratio of the volume of sample forced to flow through the chamber to the volume capacity of the chamber is at least 2:1, and wherein the volume of sample forced to flow through the chamber is at least 100  $\mu$ l;
- c) disrupting the captured cells or viruses to release the analyte therefrom; and
- d) eluting the analyte from the chamber.

16. The method of claim 15, wherein the ratio of the volume of sample forced to flow through the chamber to the volume capacity of the chamber is at least 5:1.

17. The method of claim 15, wherein the volume of sample forced to flow through the chamber is at least 1 ml.

18. The method of claim 15, wherein the step of disrupting the cells or viruses comprises agitating beads in the chamber.

19. The method of claim 15, wherein step of disrupting the cells or viruses comprises sonicating the chamber.

20. The method of claim 15, wherein step of disrupting the cells or viruses comprises heating the chamber.

21. The method of claim 15, further comprising the step of sonicating the chamber while forcing the sample to flow through the chamber.

22. The method of claim 15, wherein the step of eluting the analyte is preceded by the step of forcing a wash fluid to flow through the chamber while sonicating the chamber.

5

23. The method of claim 15, further comprising the step of sonicating the chamber while eluting the analyte.

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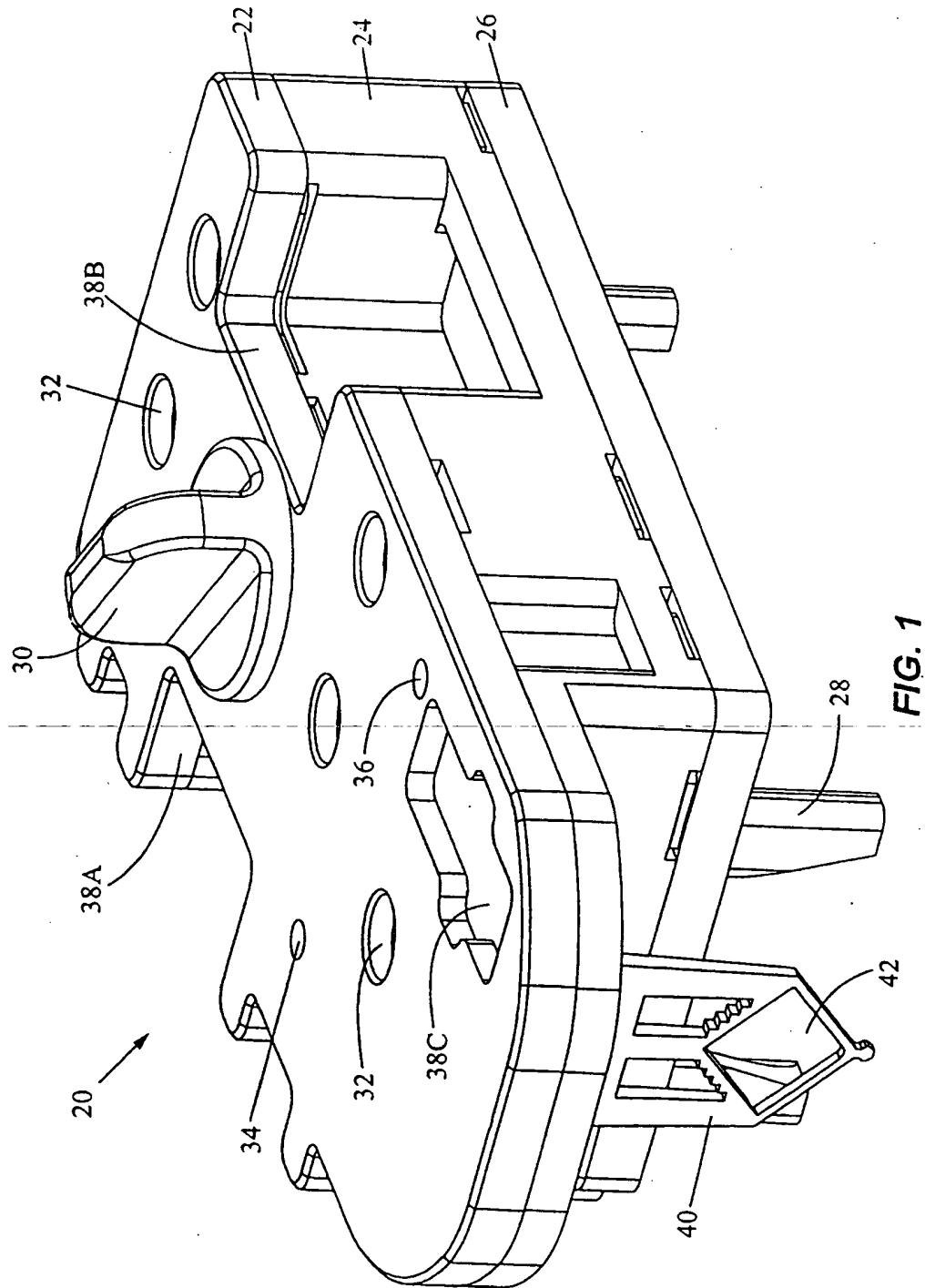


FIG. 1

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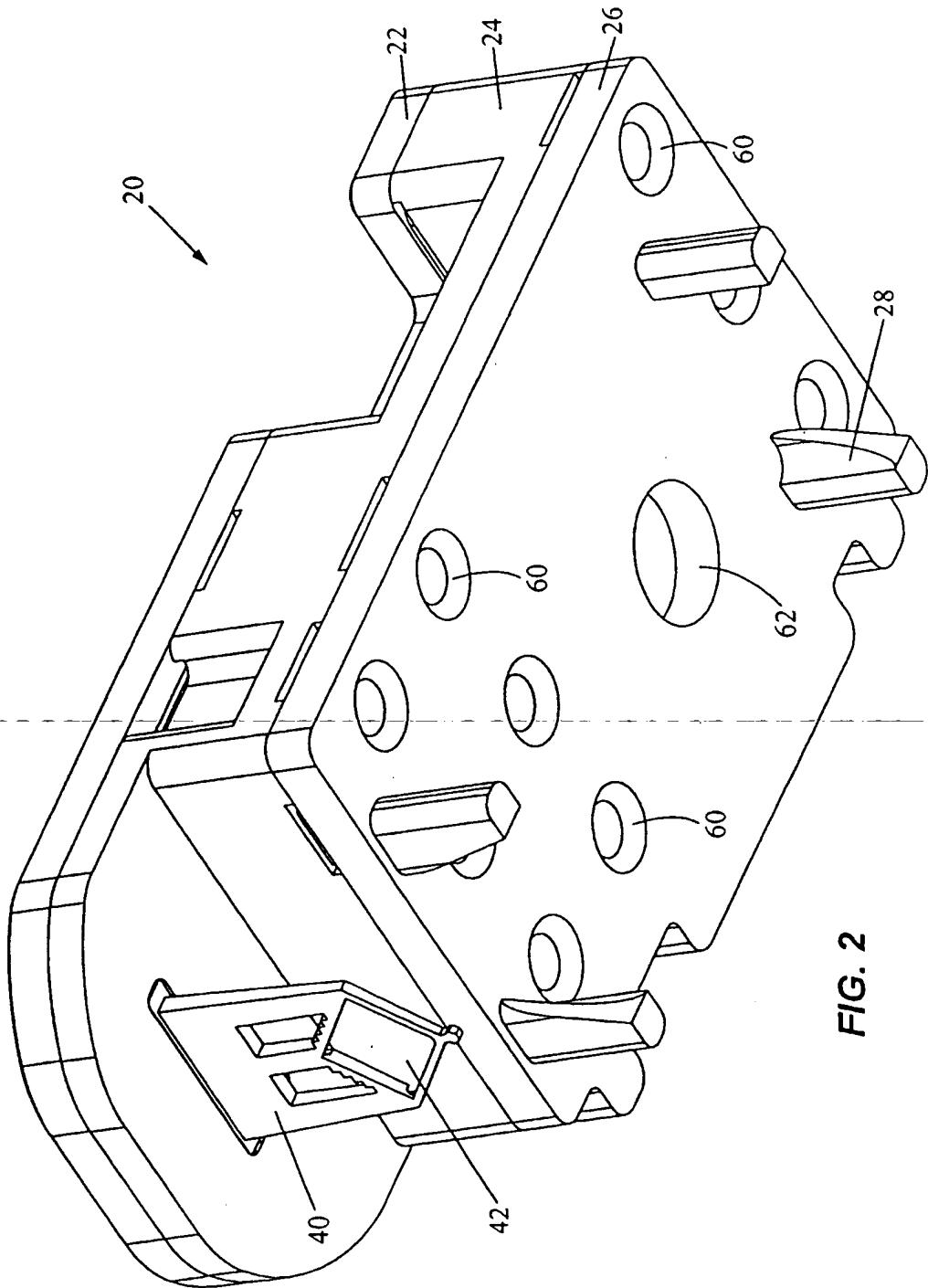


FIG. 2

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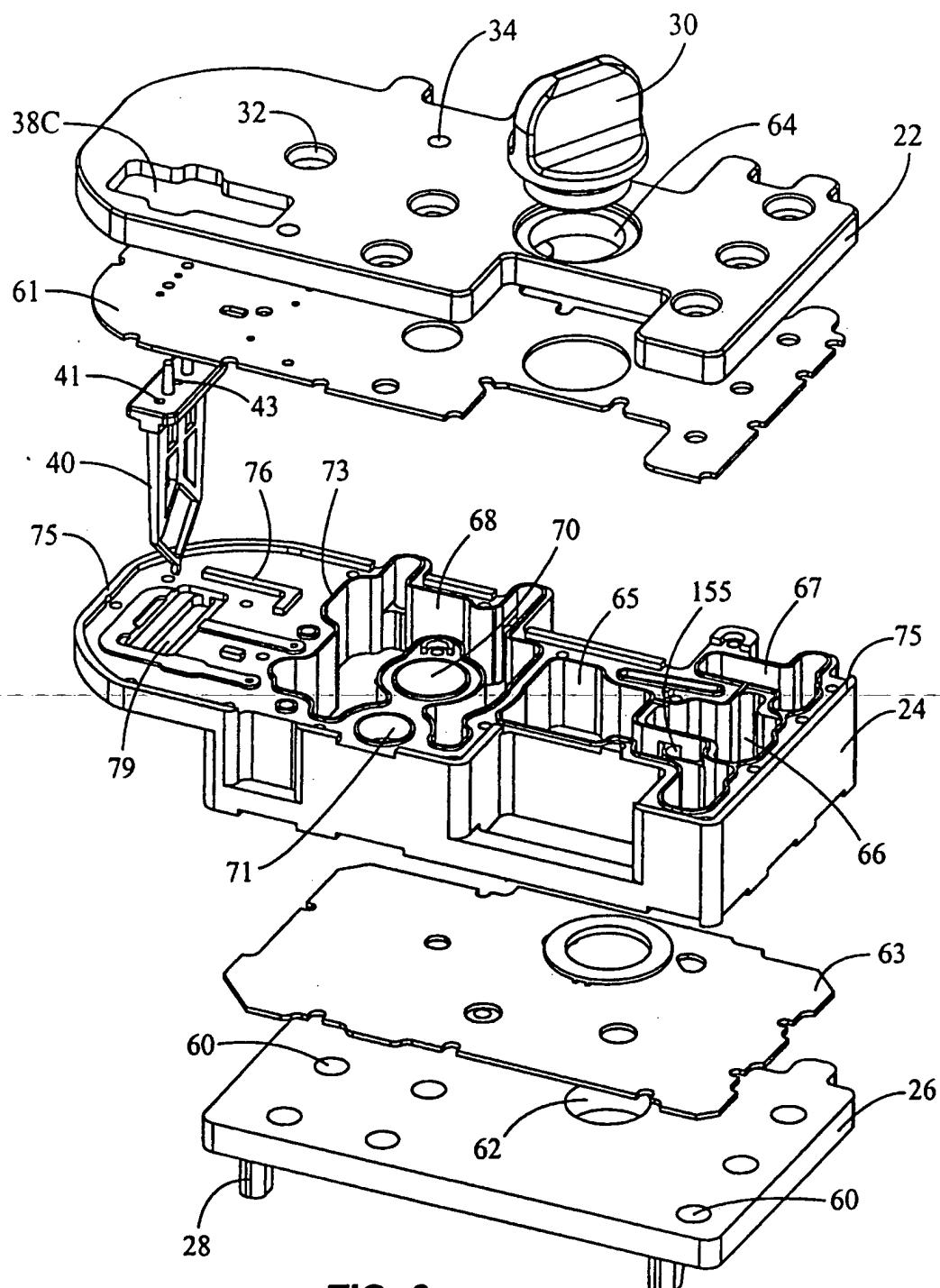


FIG. 3

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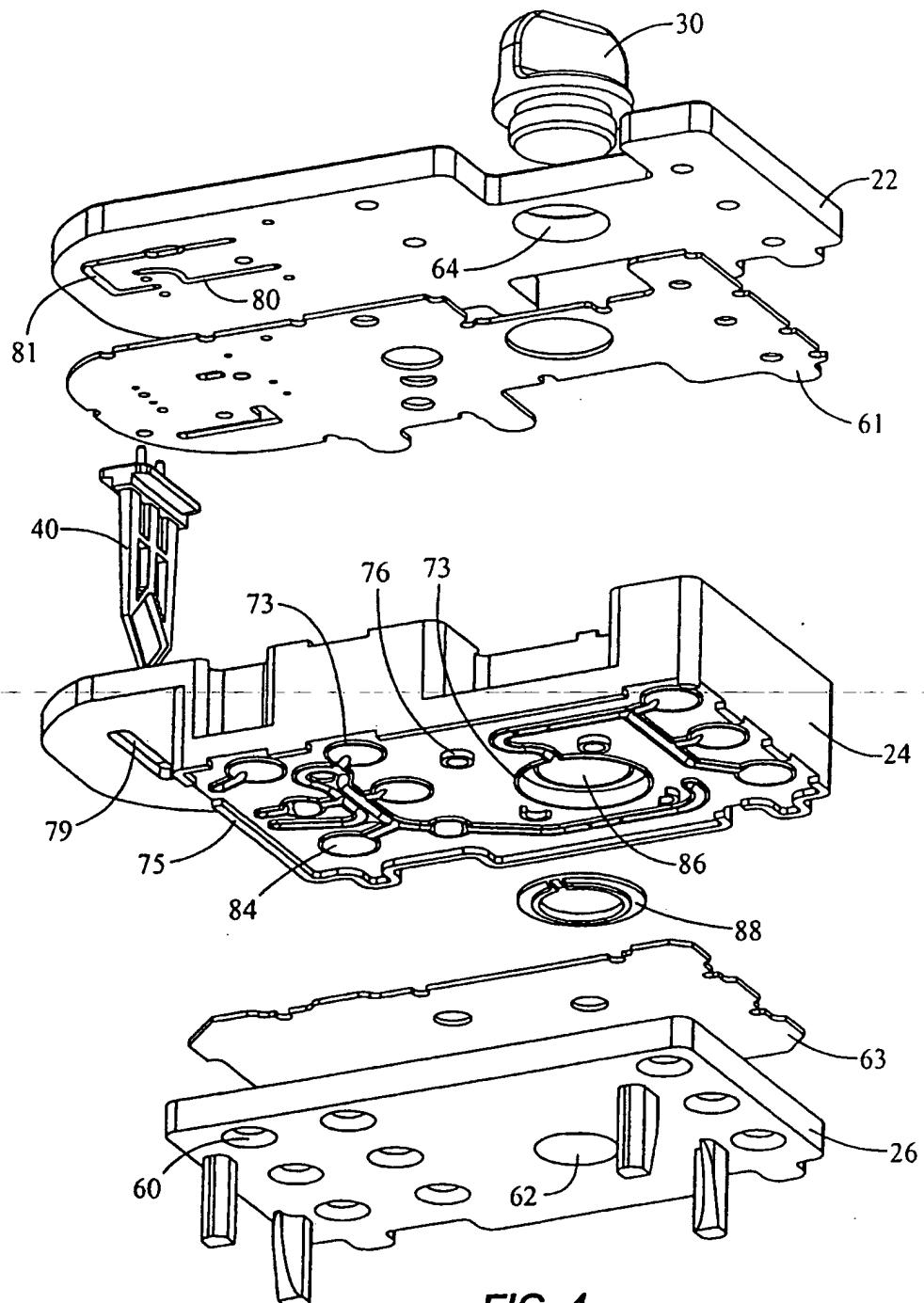
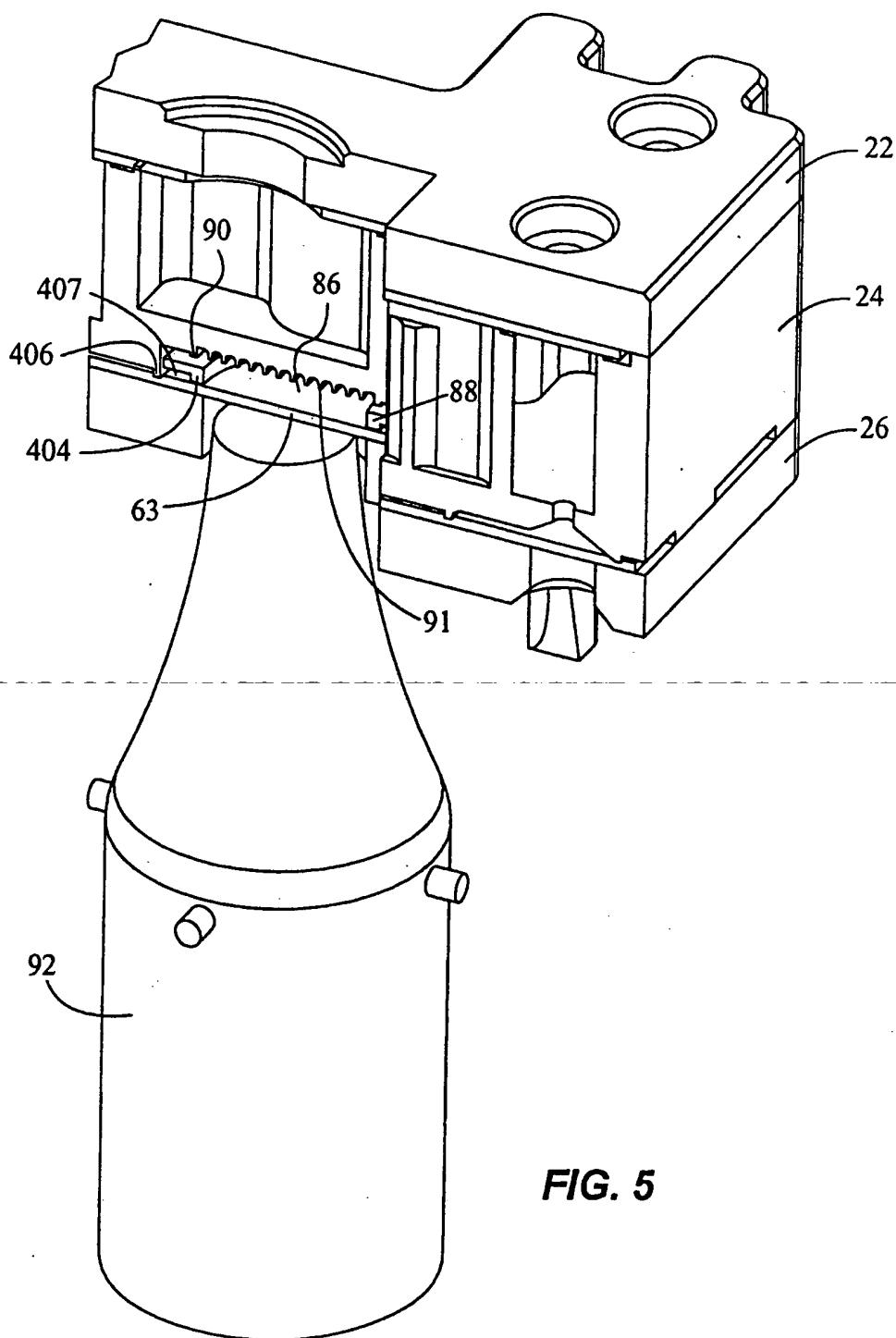


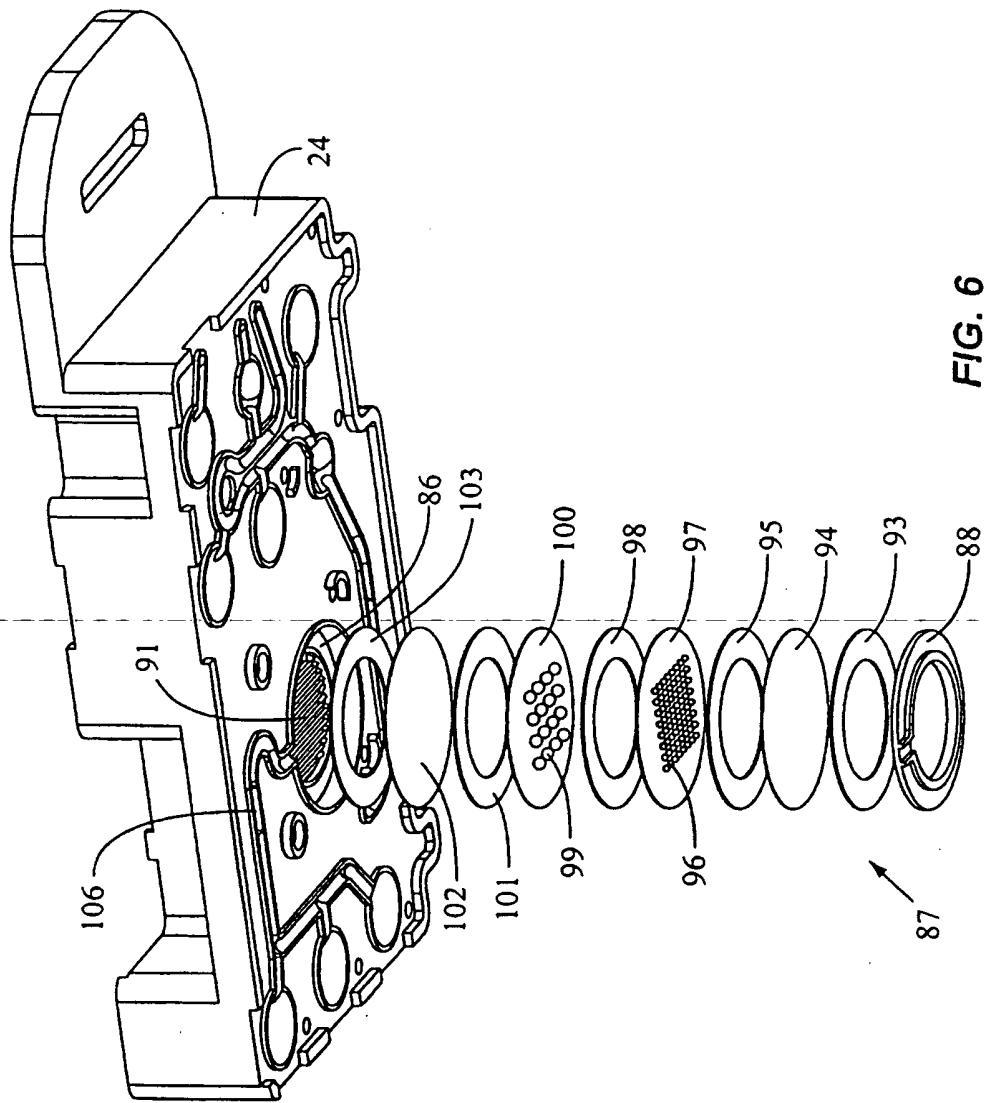
FIG. 4

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FIG. 6



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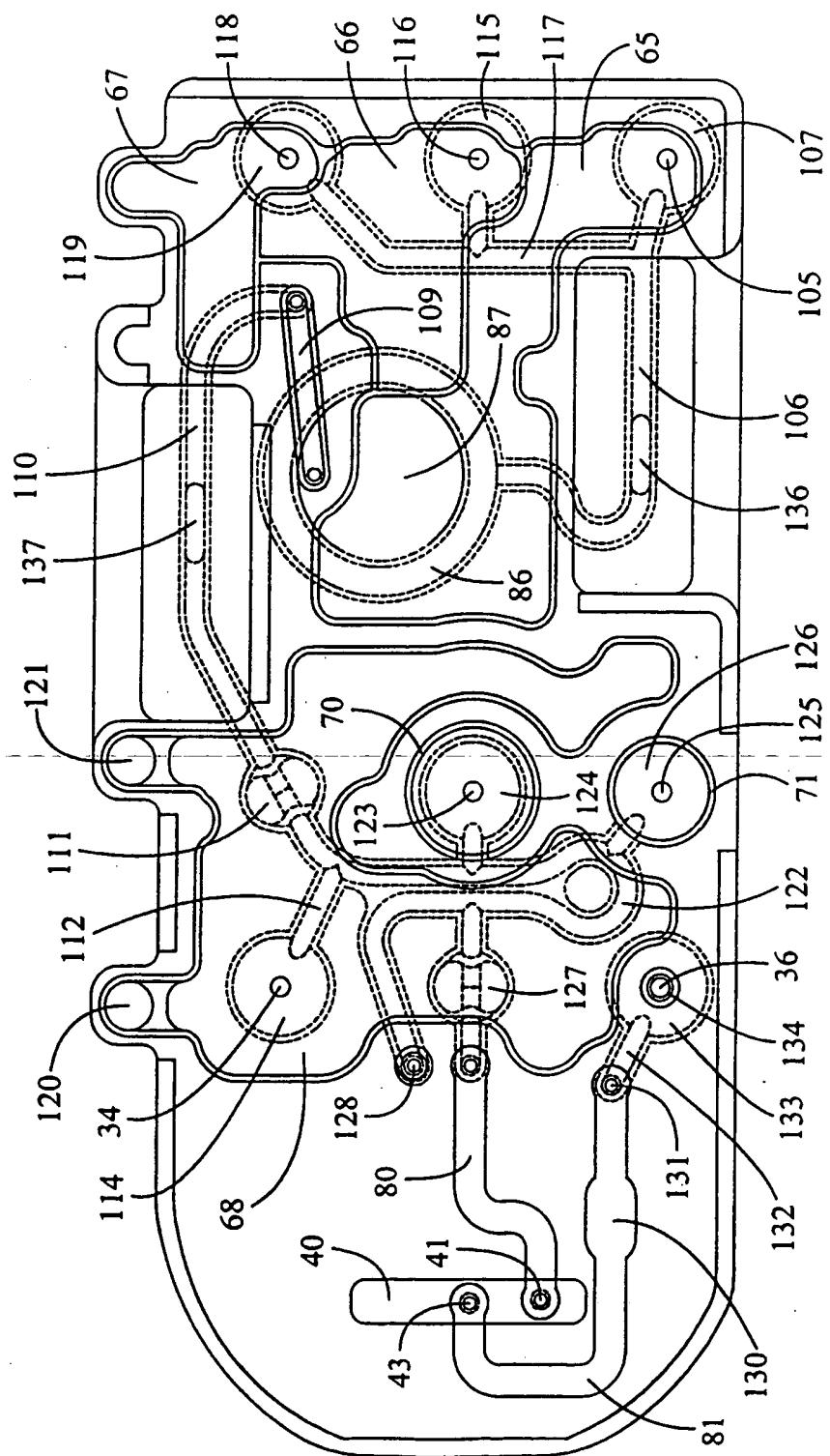


FIG. 7

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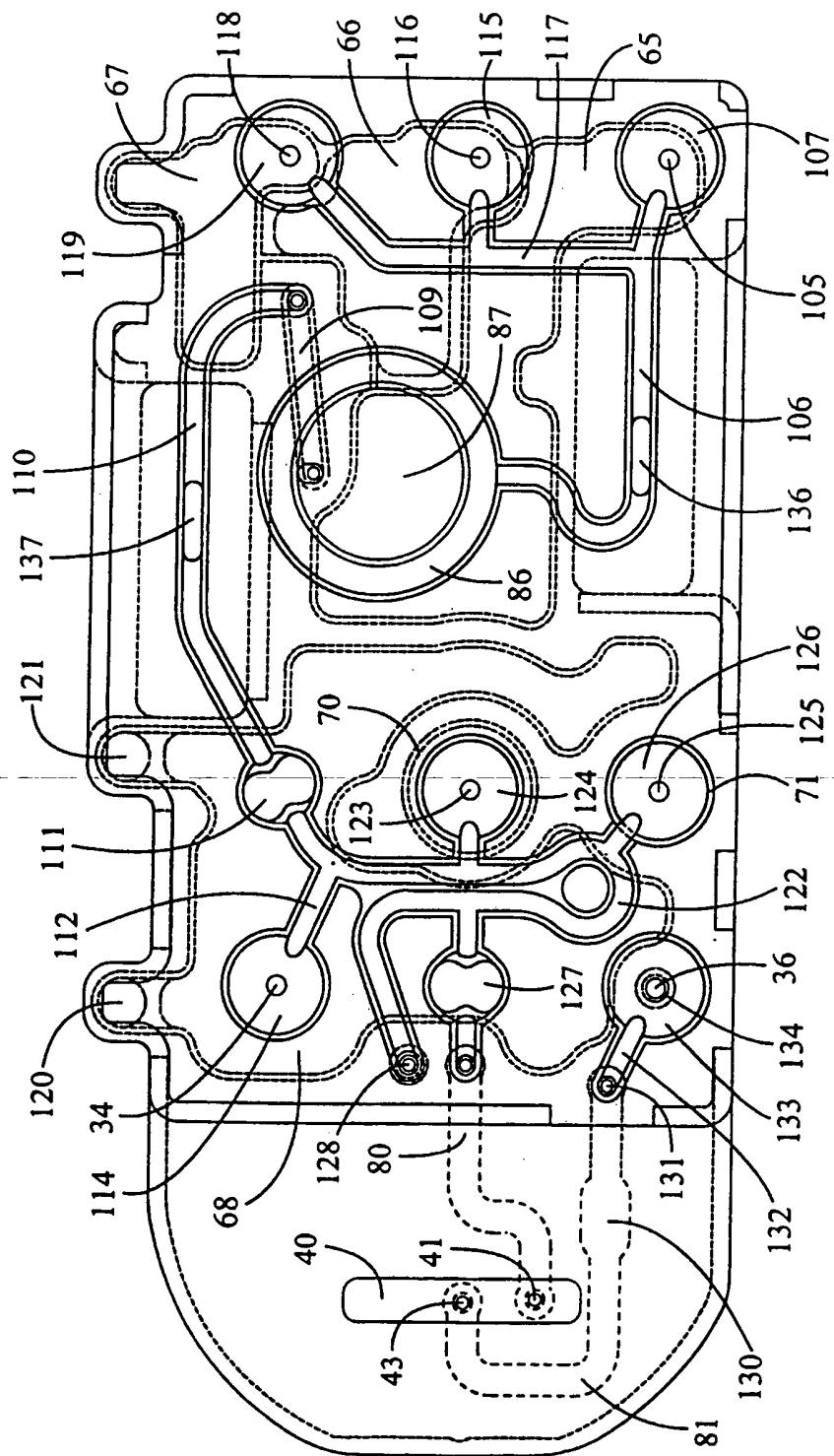


FIG. 8

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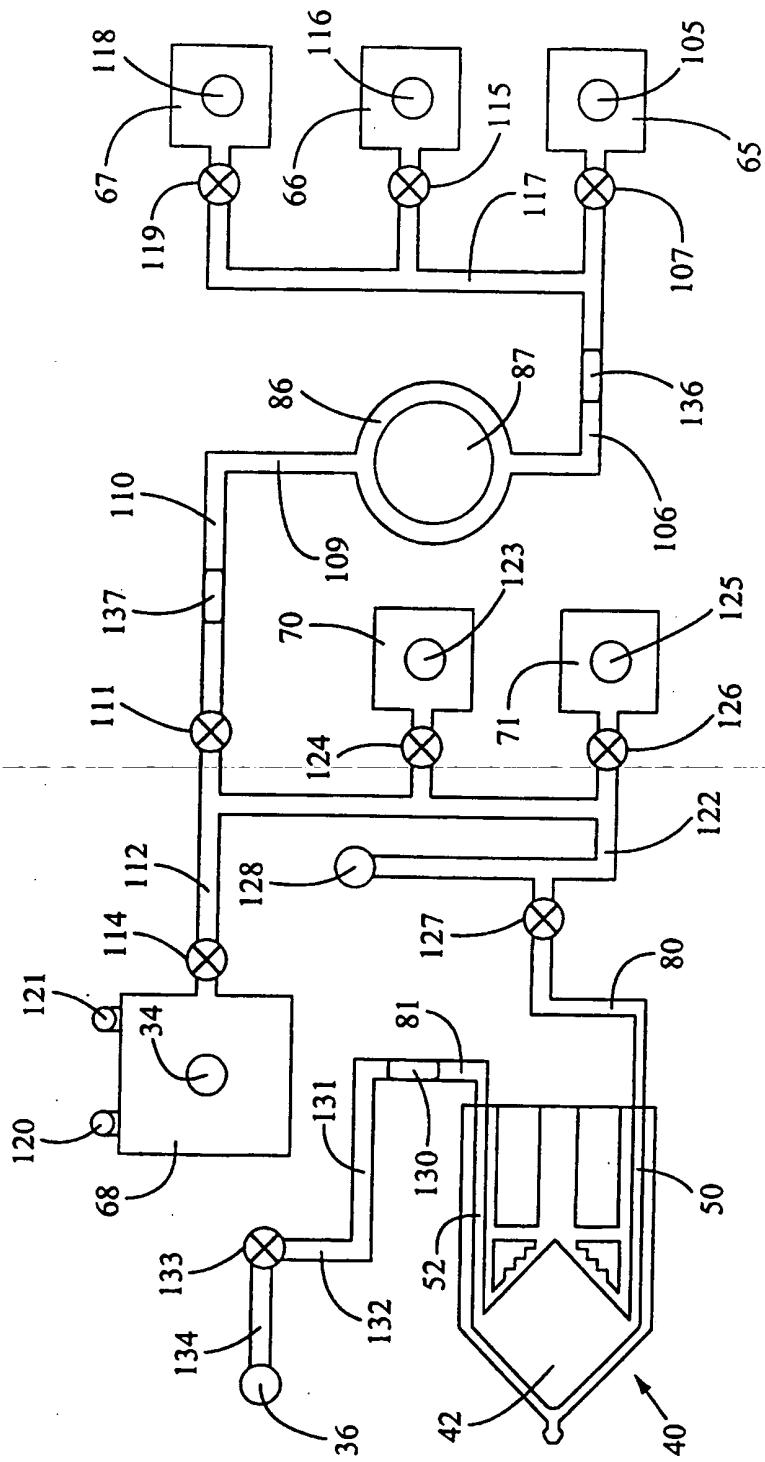


FIG. 9

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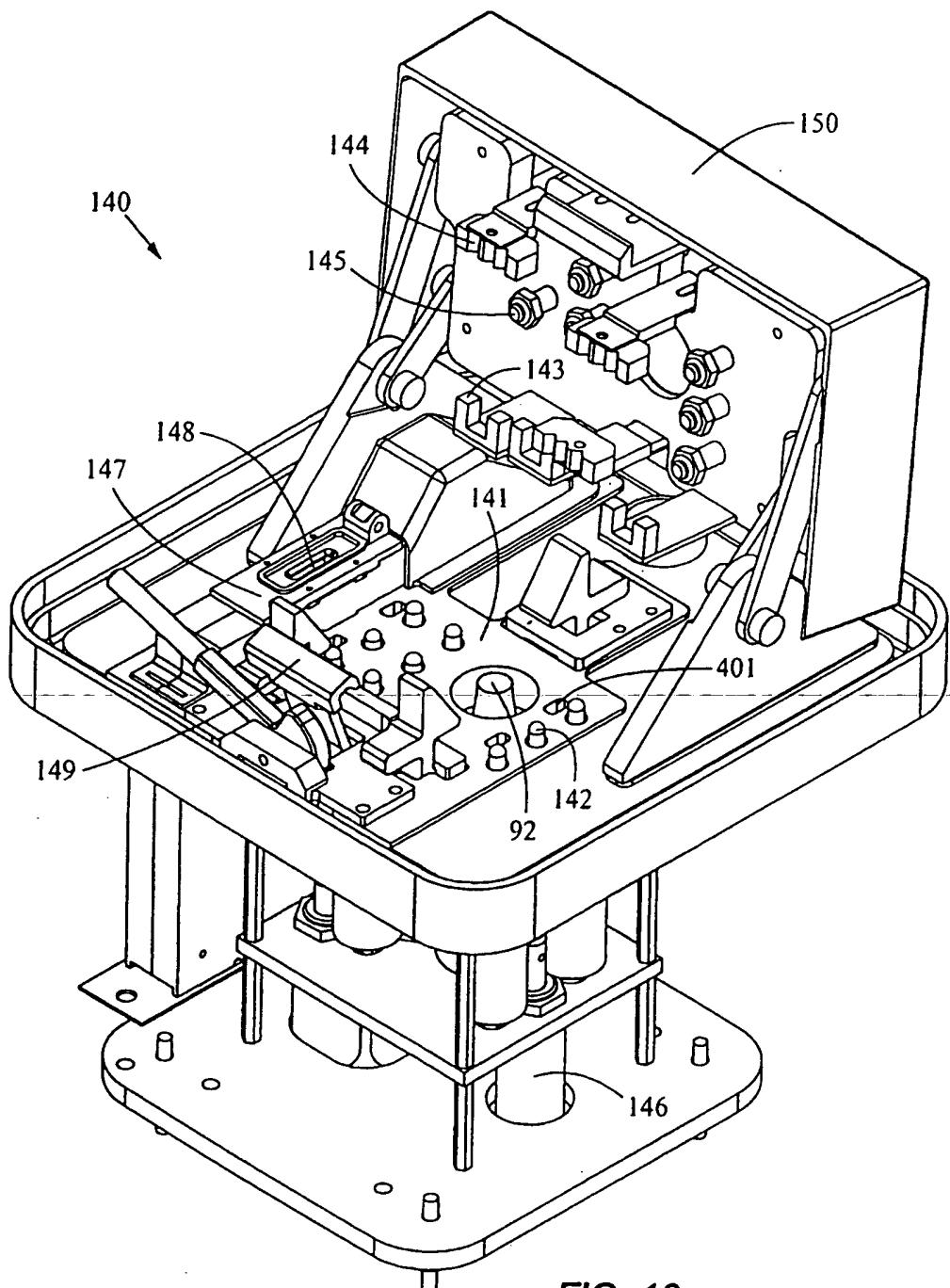


FIG. 10

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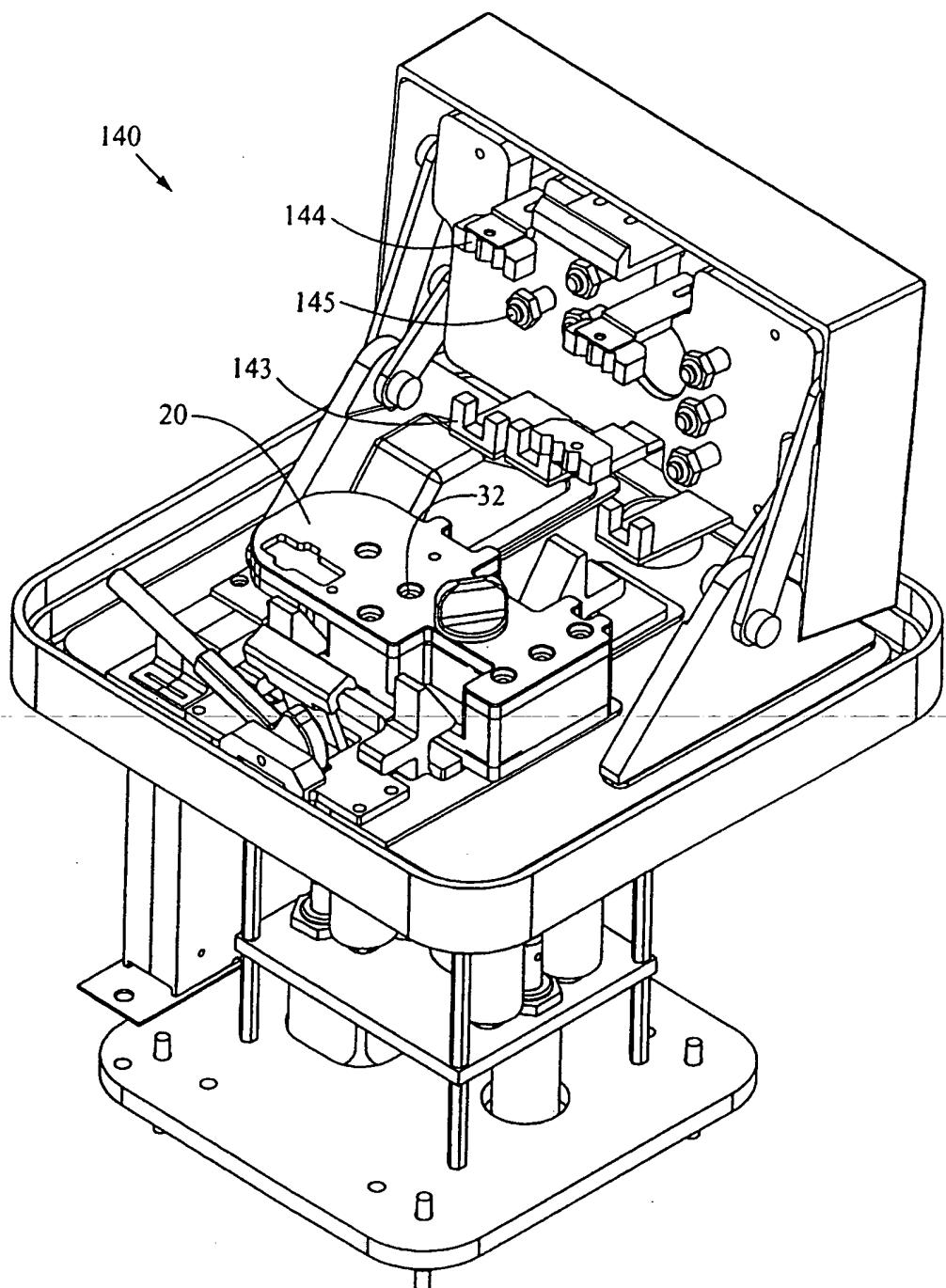
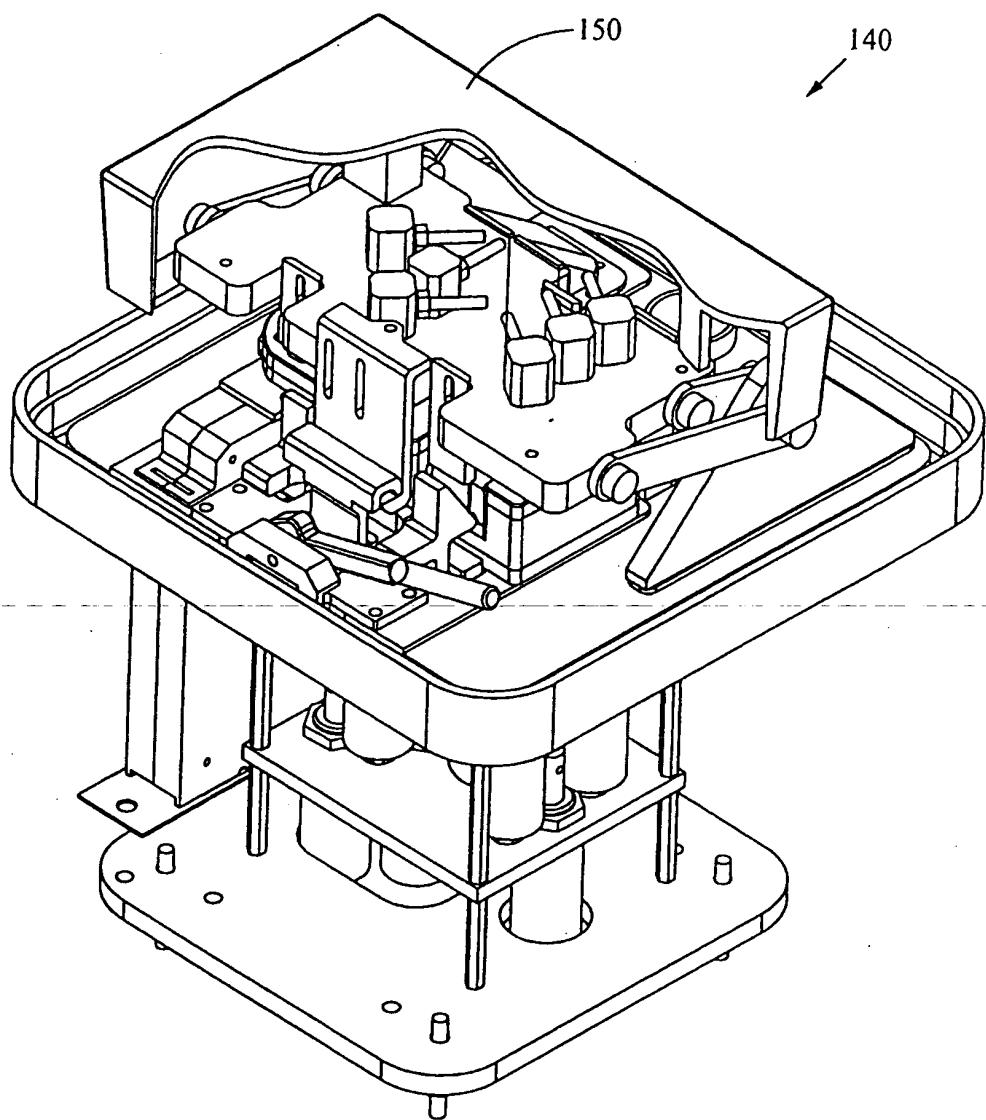


FIG. 11

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*FIG. 12*

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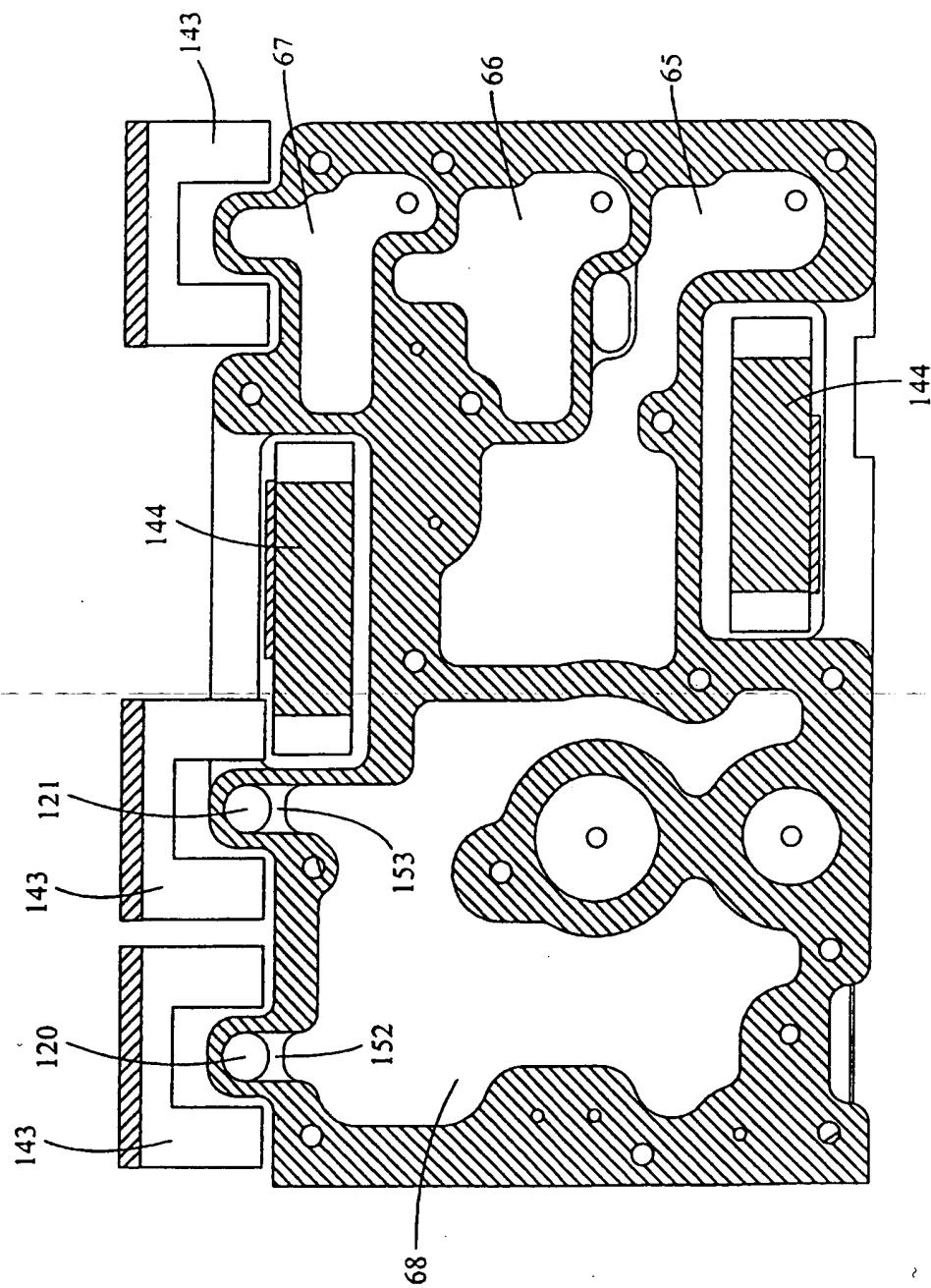
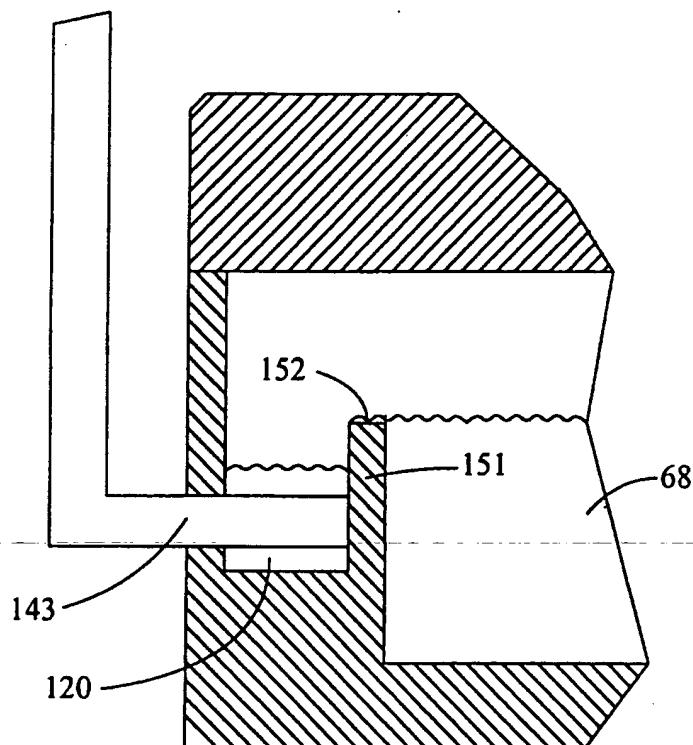


FIG. 13

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**FIG. 14**

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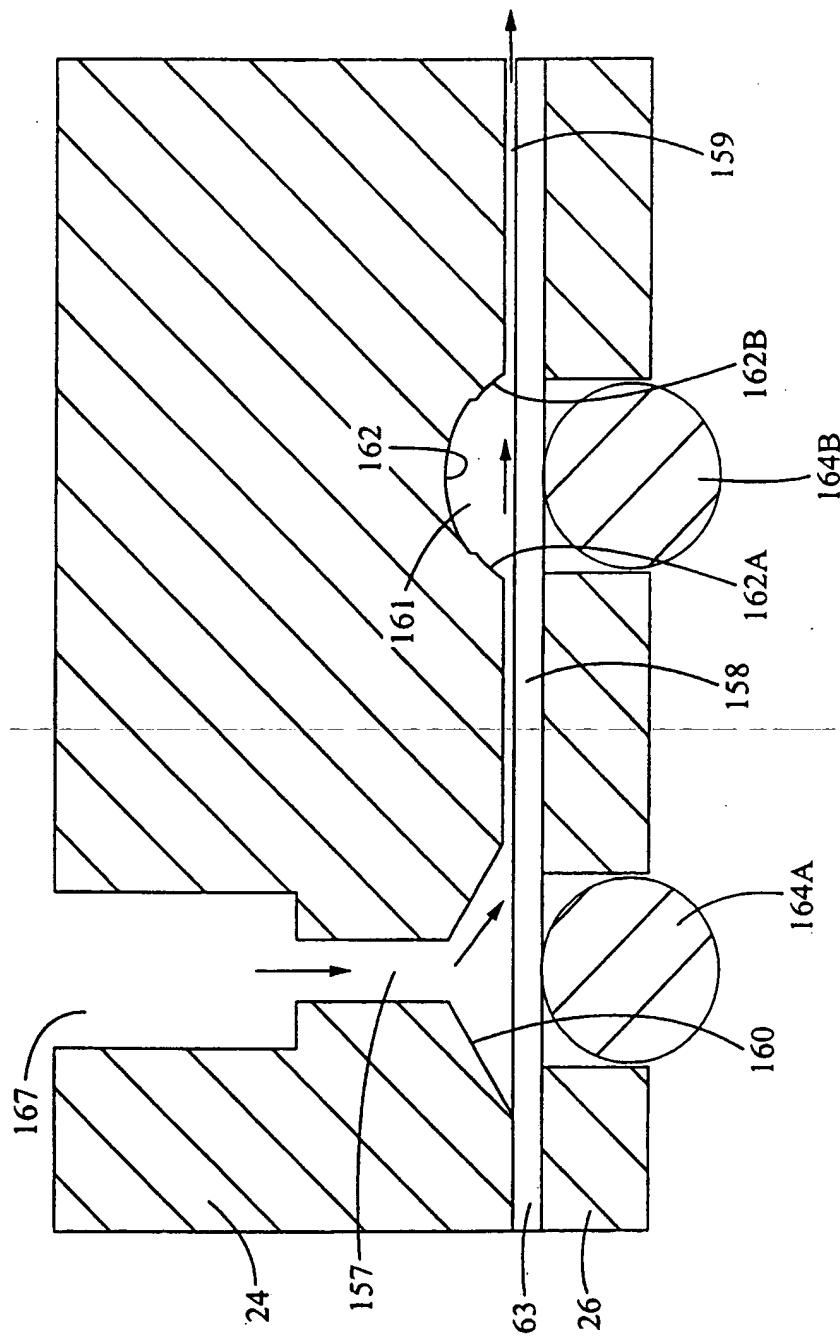
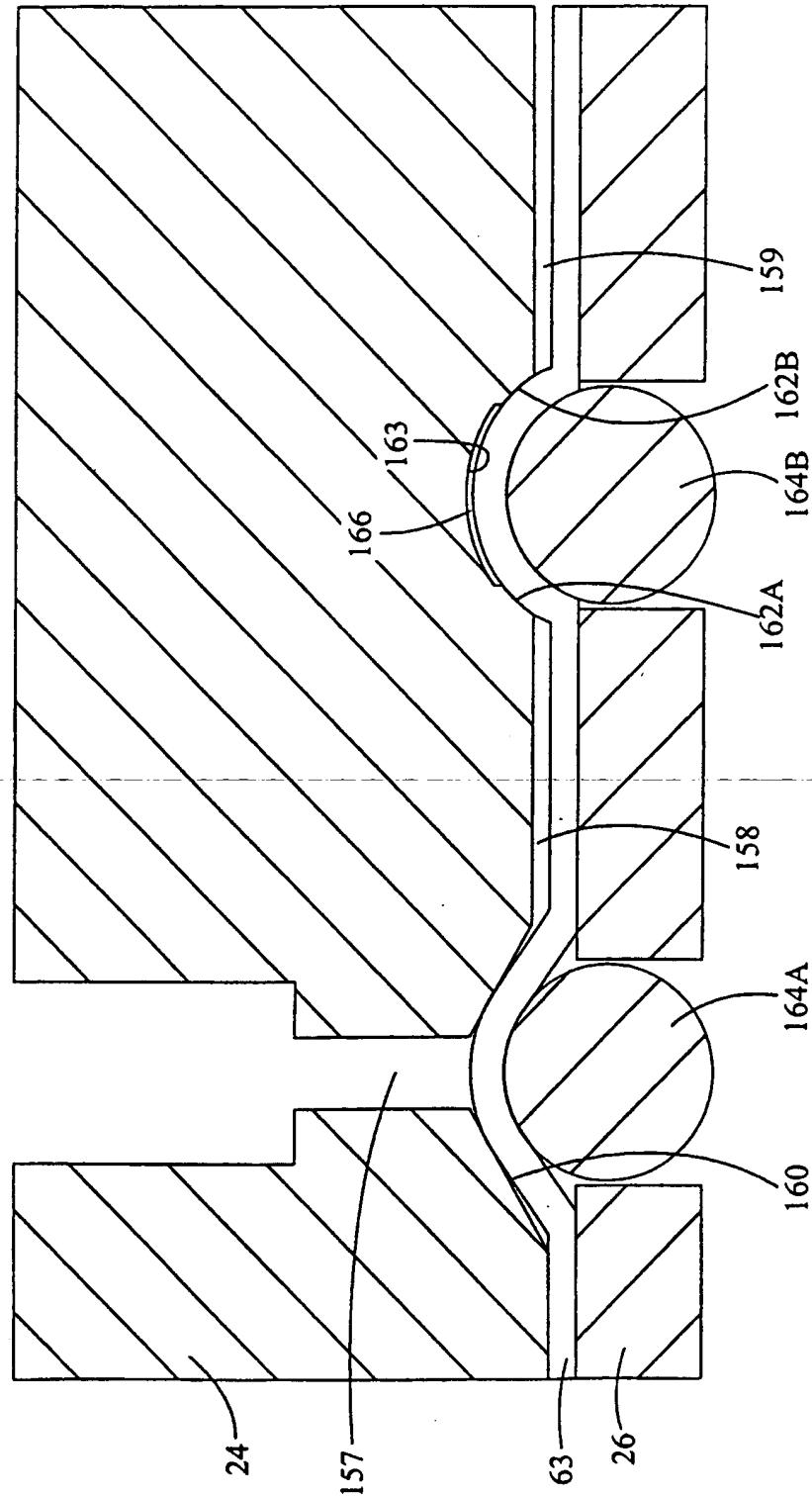


FIG. 15A

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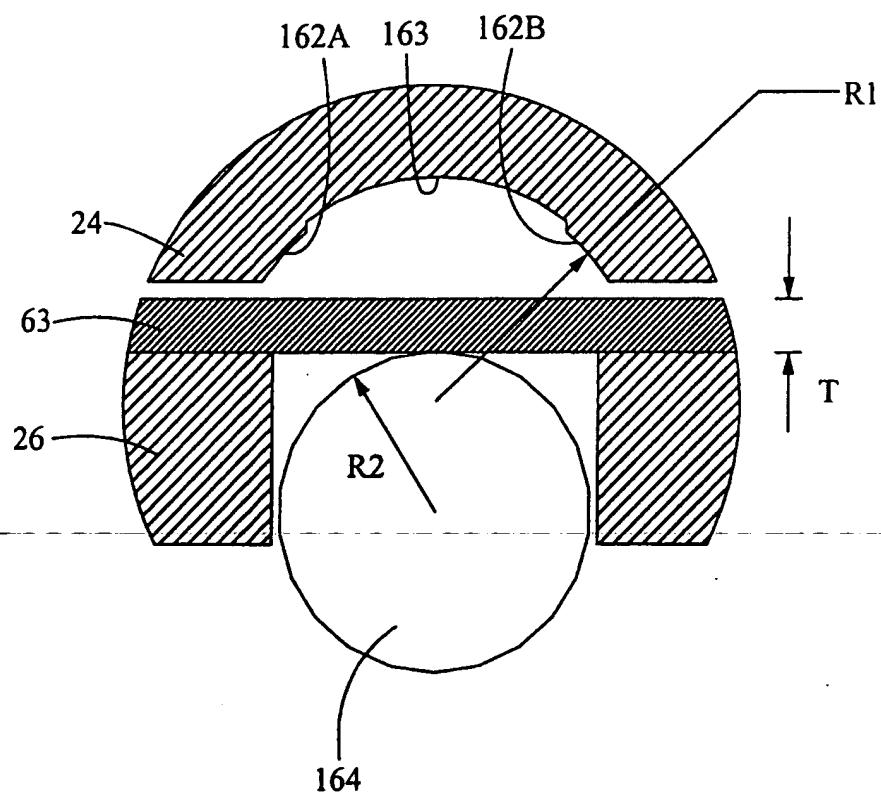


FIG. 16A

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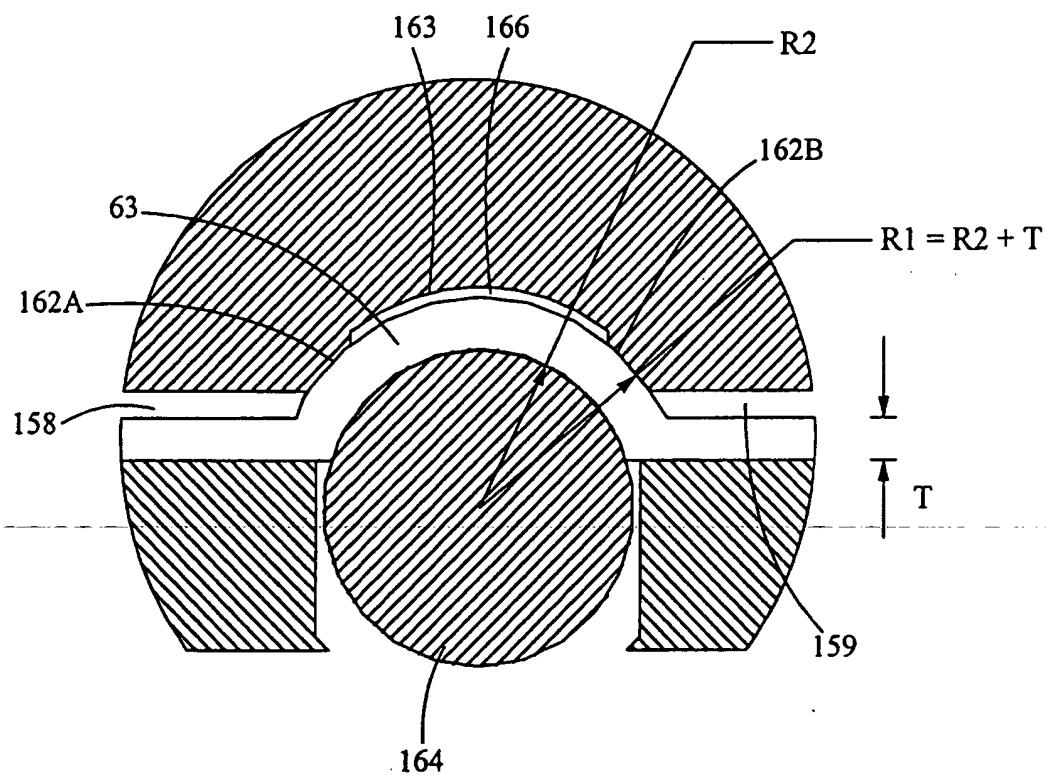


FIG. 16B

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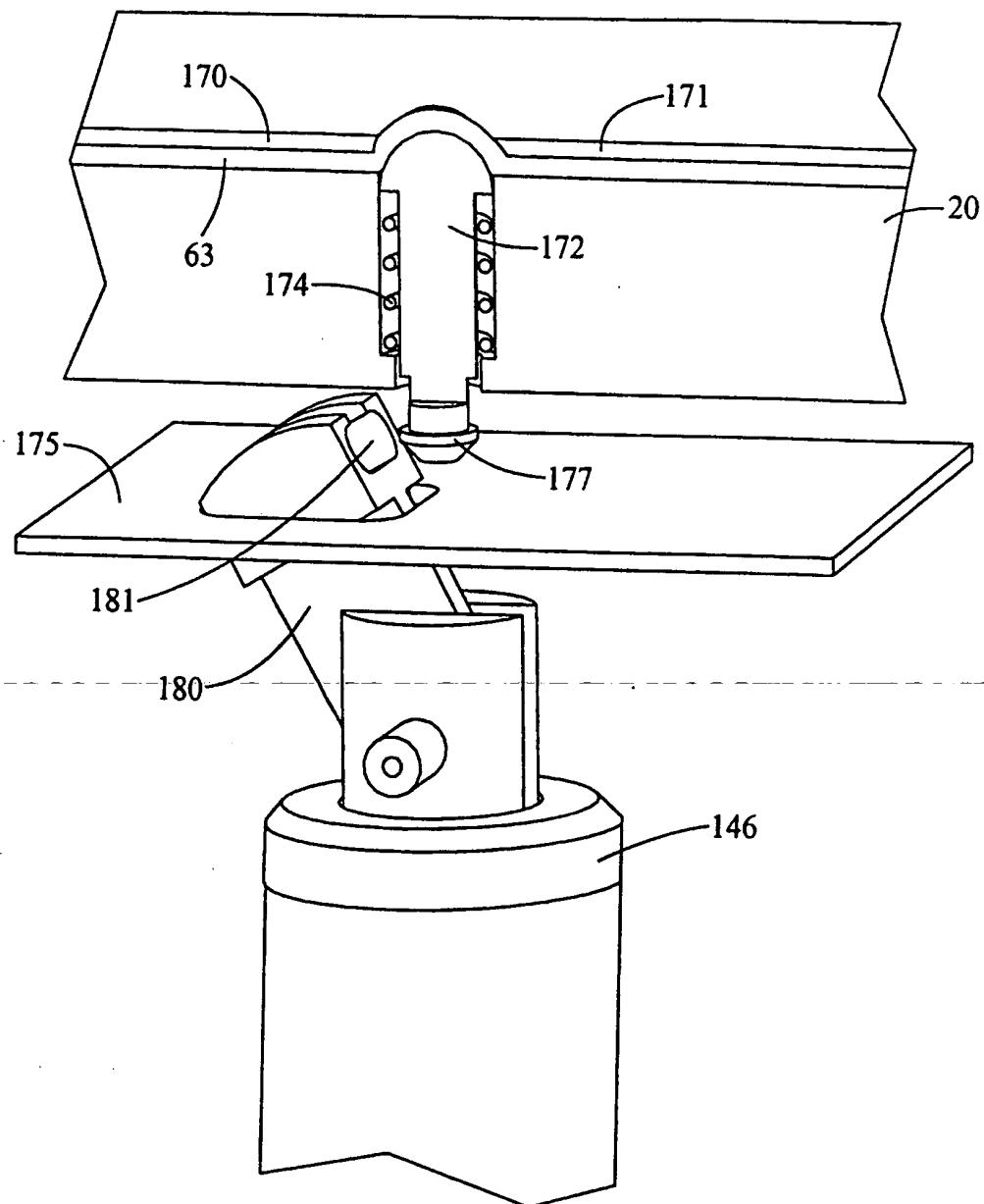
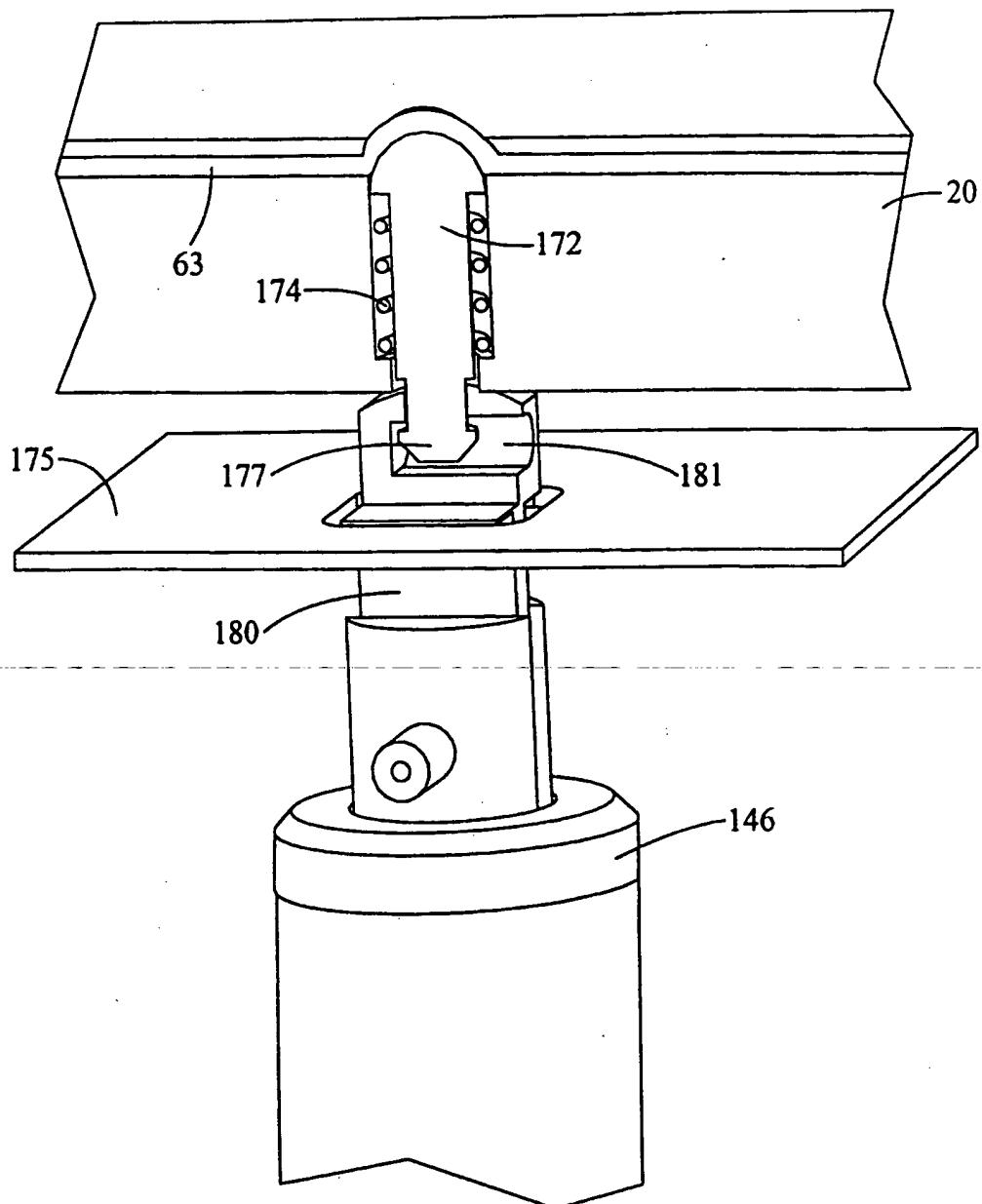


FIG. 17

**20/39****FIG. 18**

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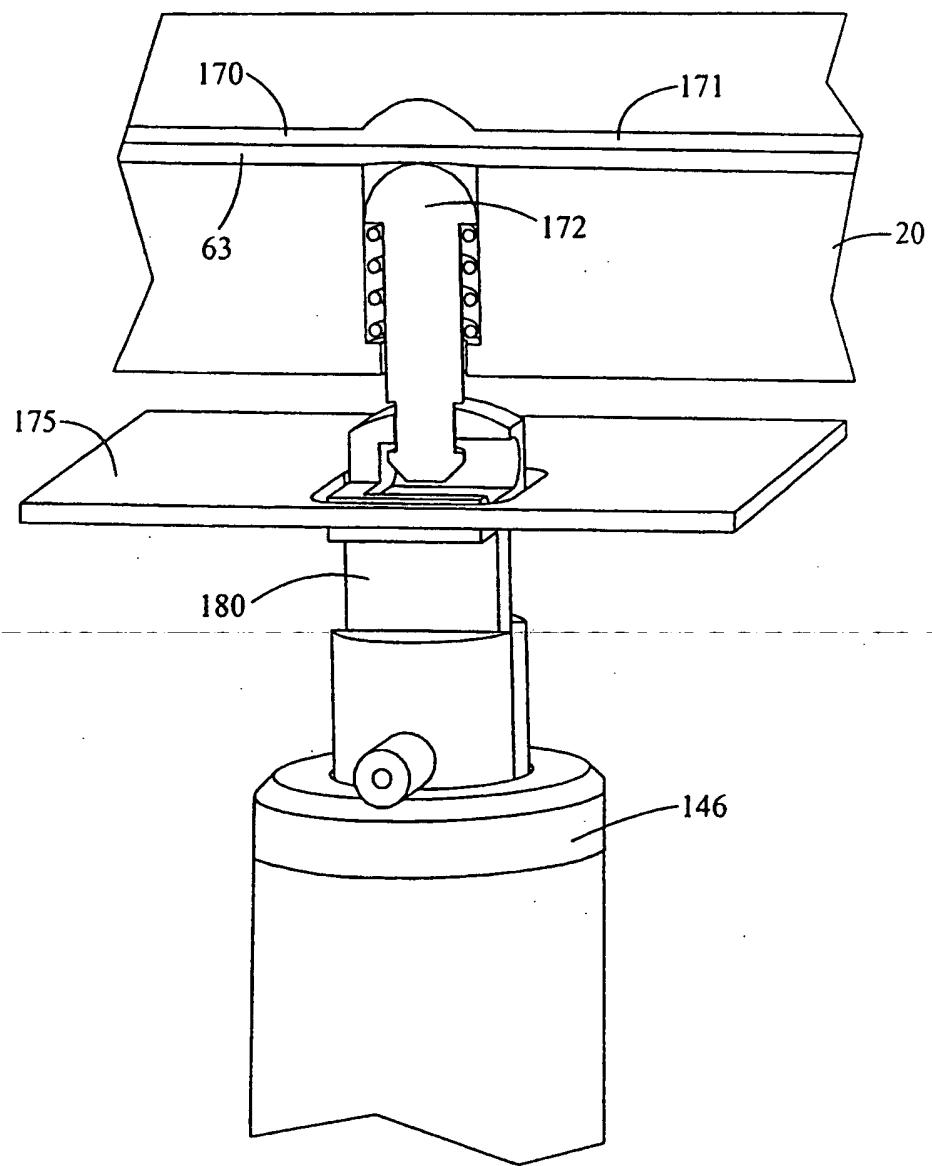


FIG. 19

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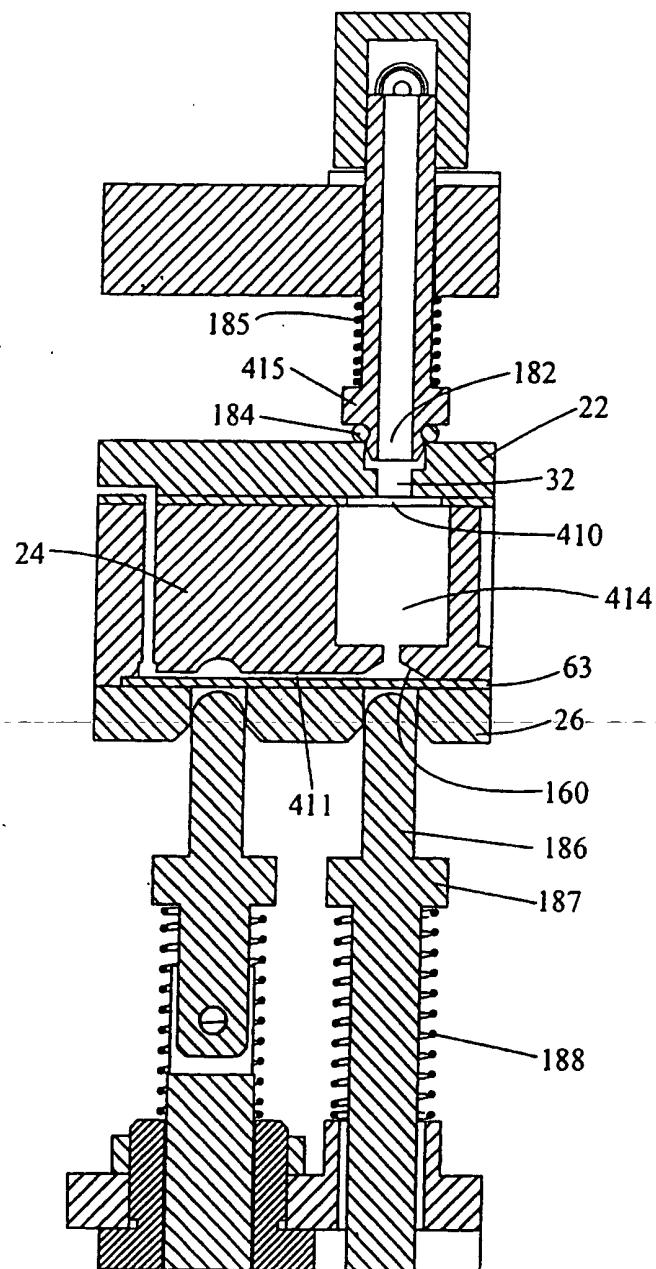


FIG. 20

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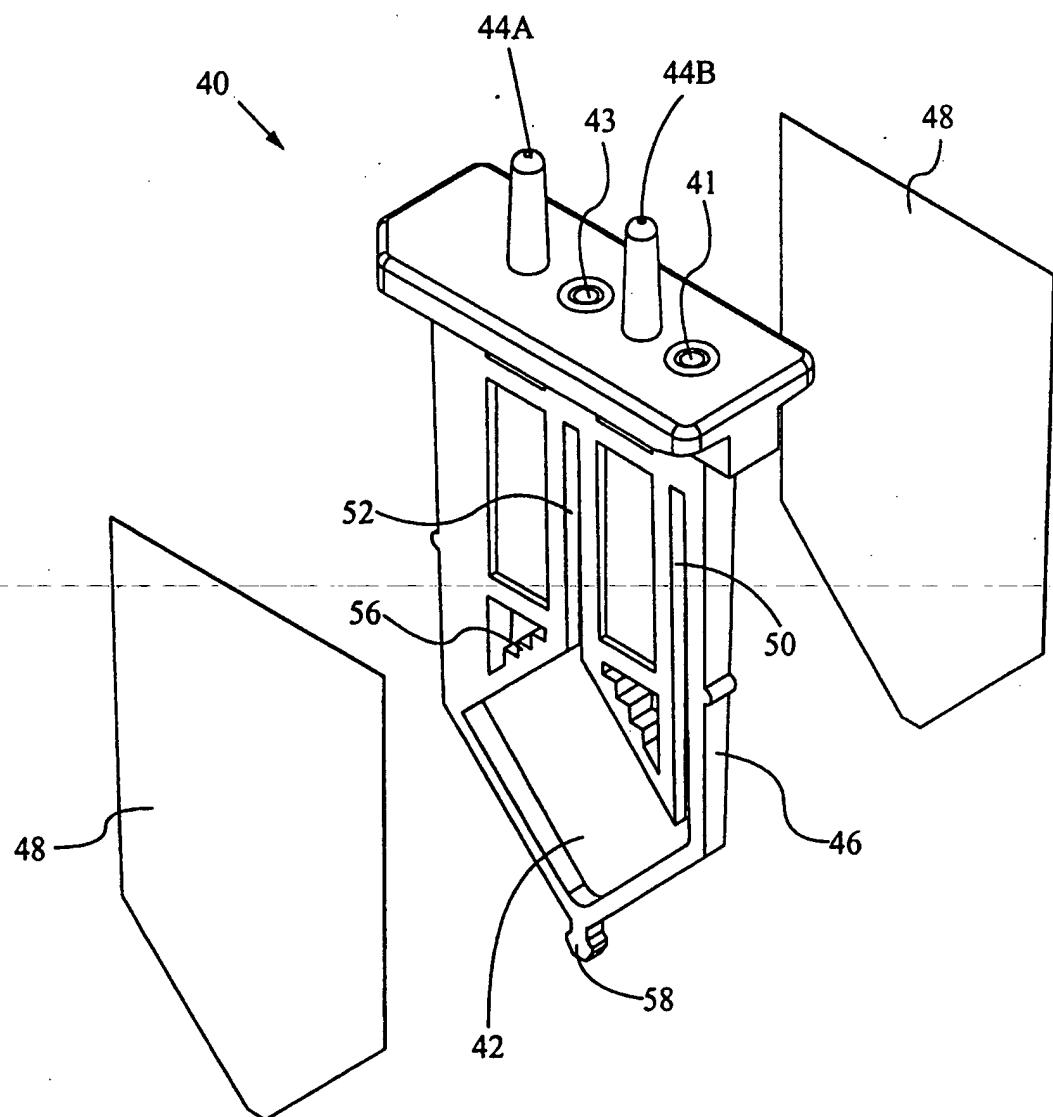


FIG. 21

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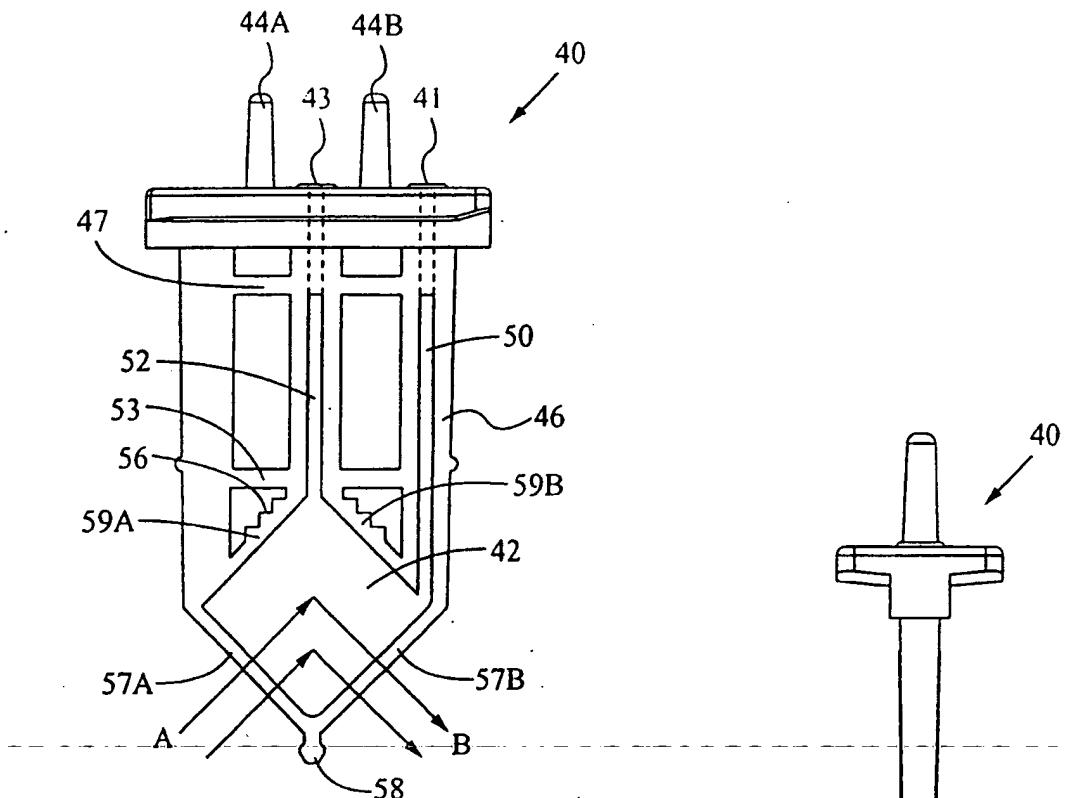


FIG. 22

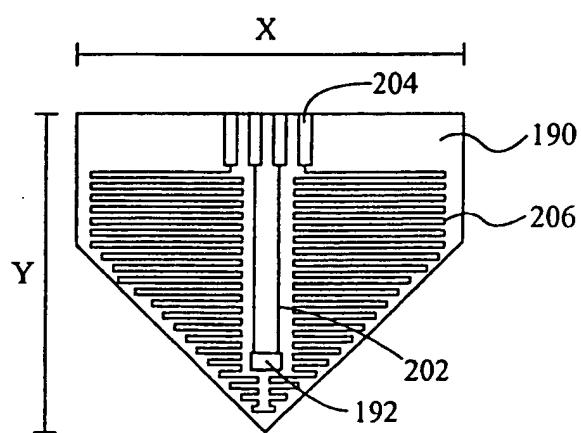


FIG. 24

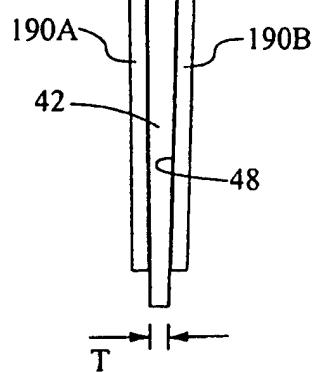
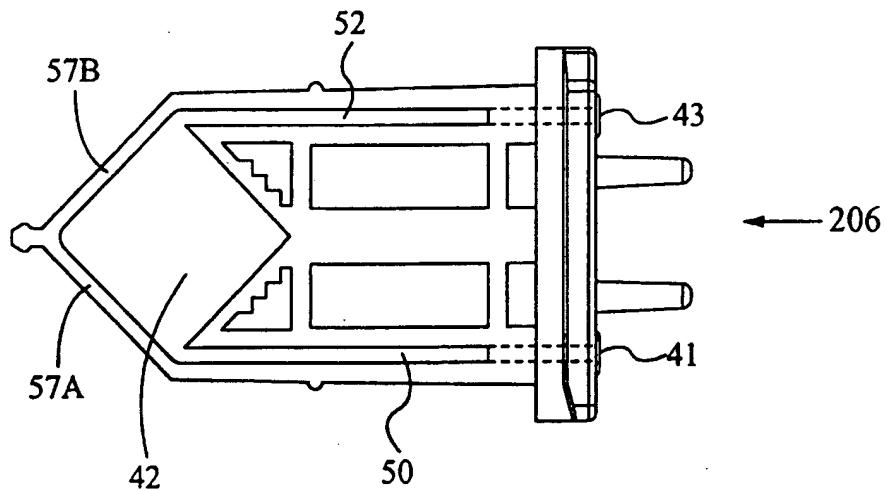
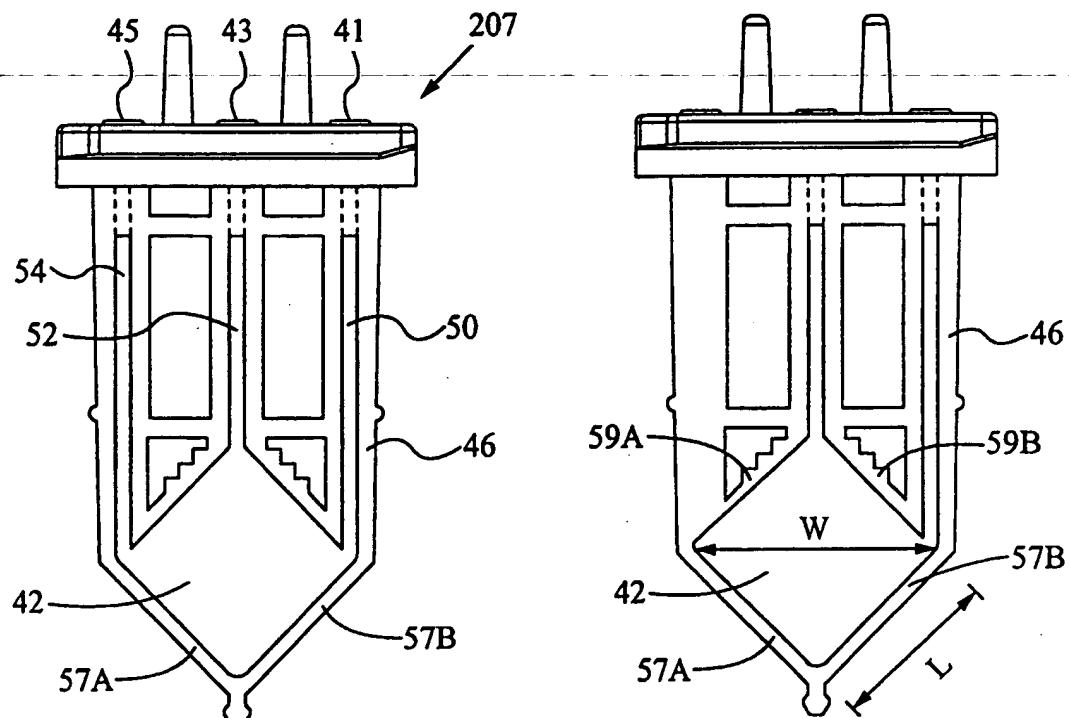


FIG. 23

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**FIG. 25**



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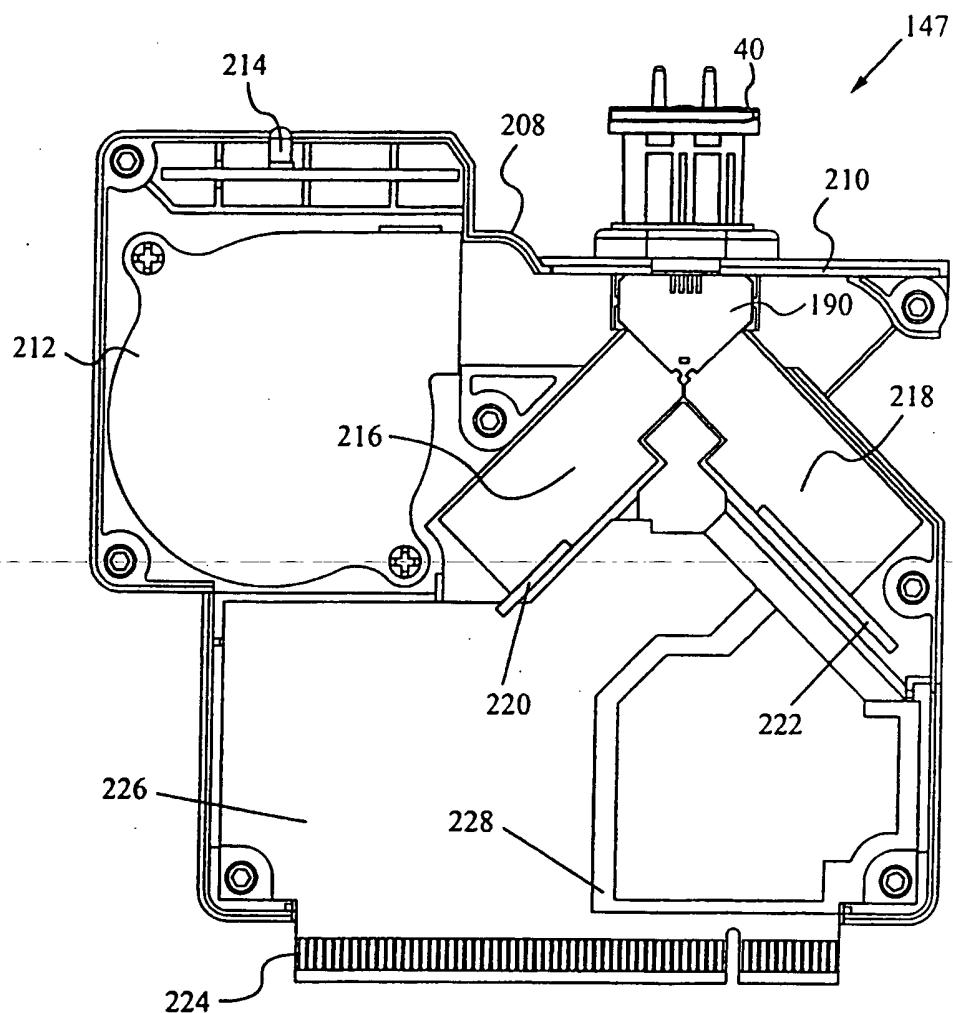


FIG. 28

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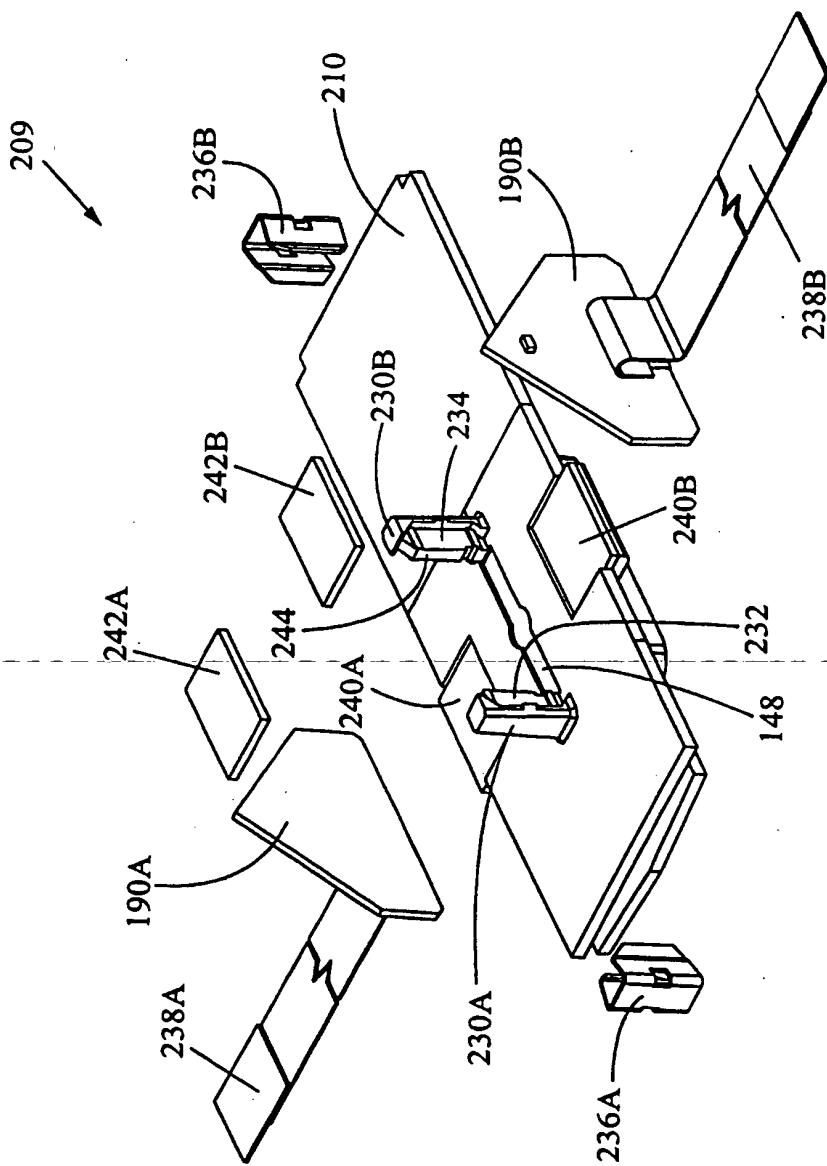
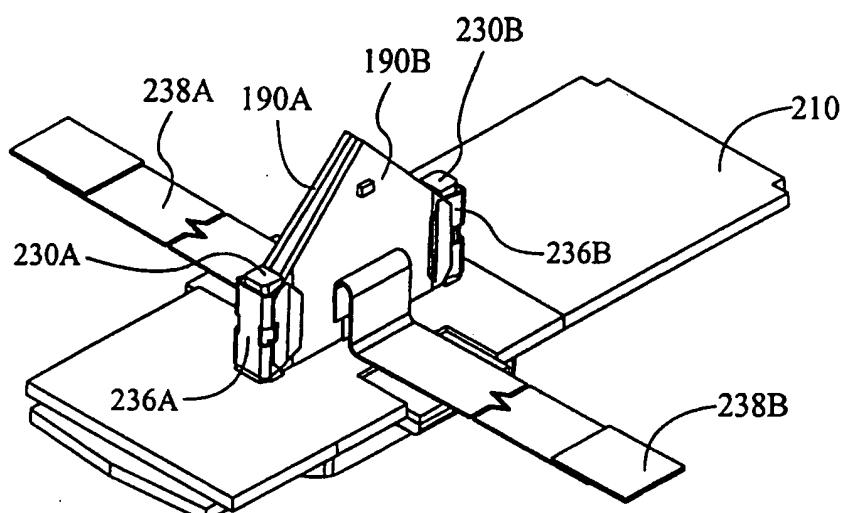
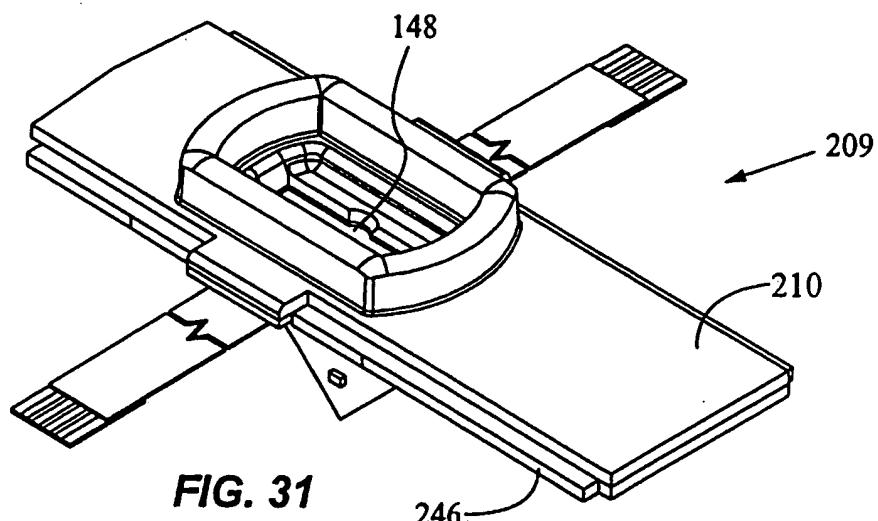
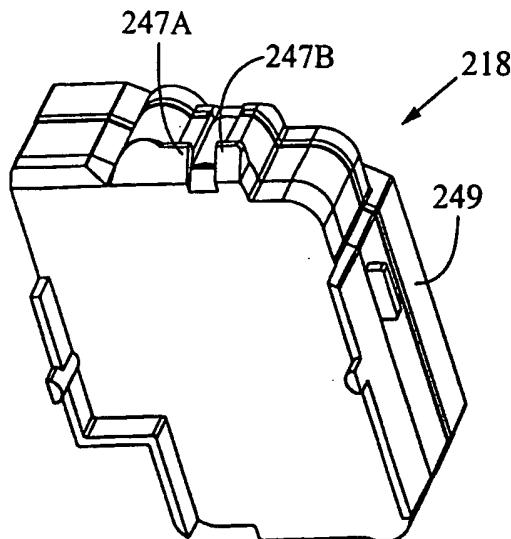
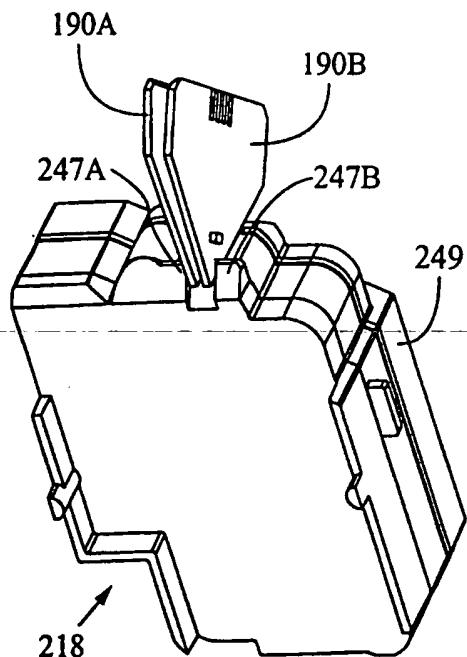
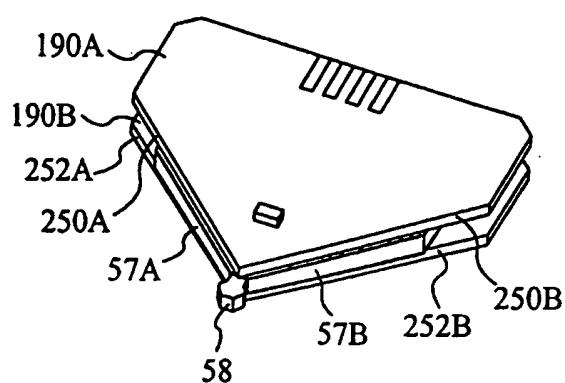


FIG. 29

**28/39****FIG. 30****FIG. 31**

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**FIG. 32****FIG. 33****FIG. 34**

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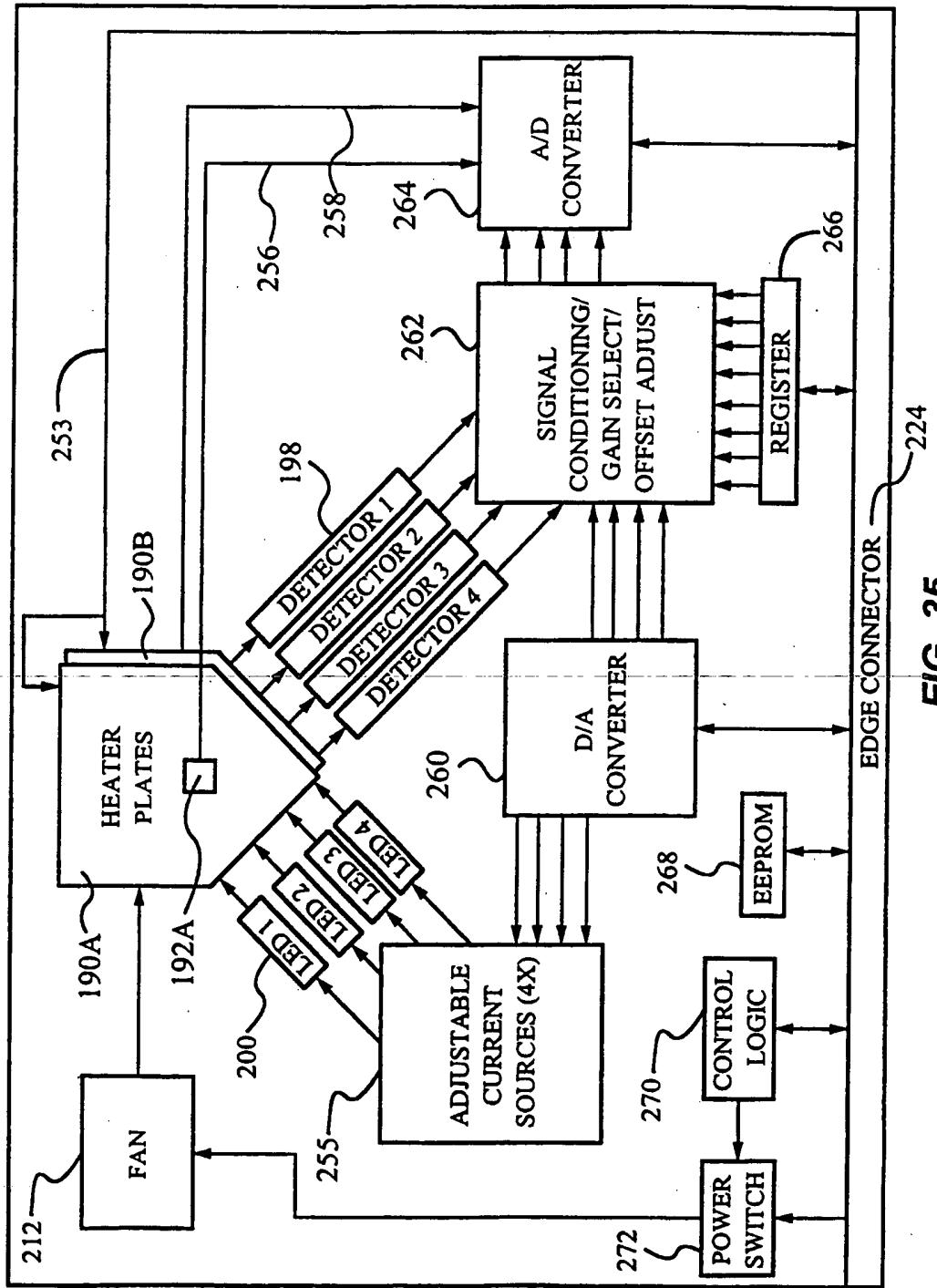


FIG. 35

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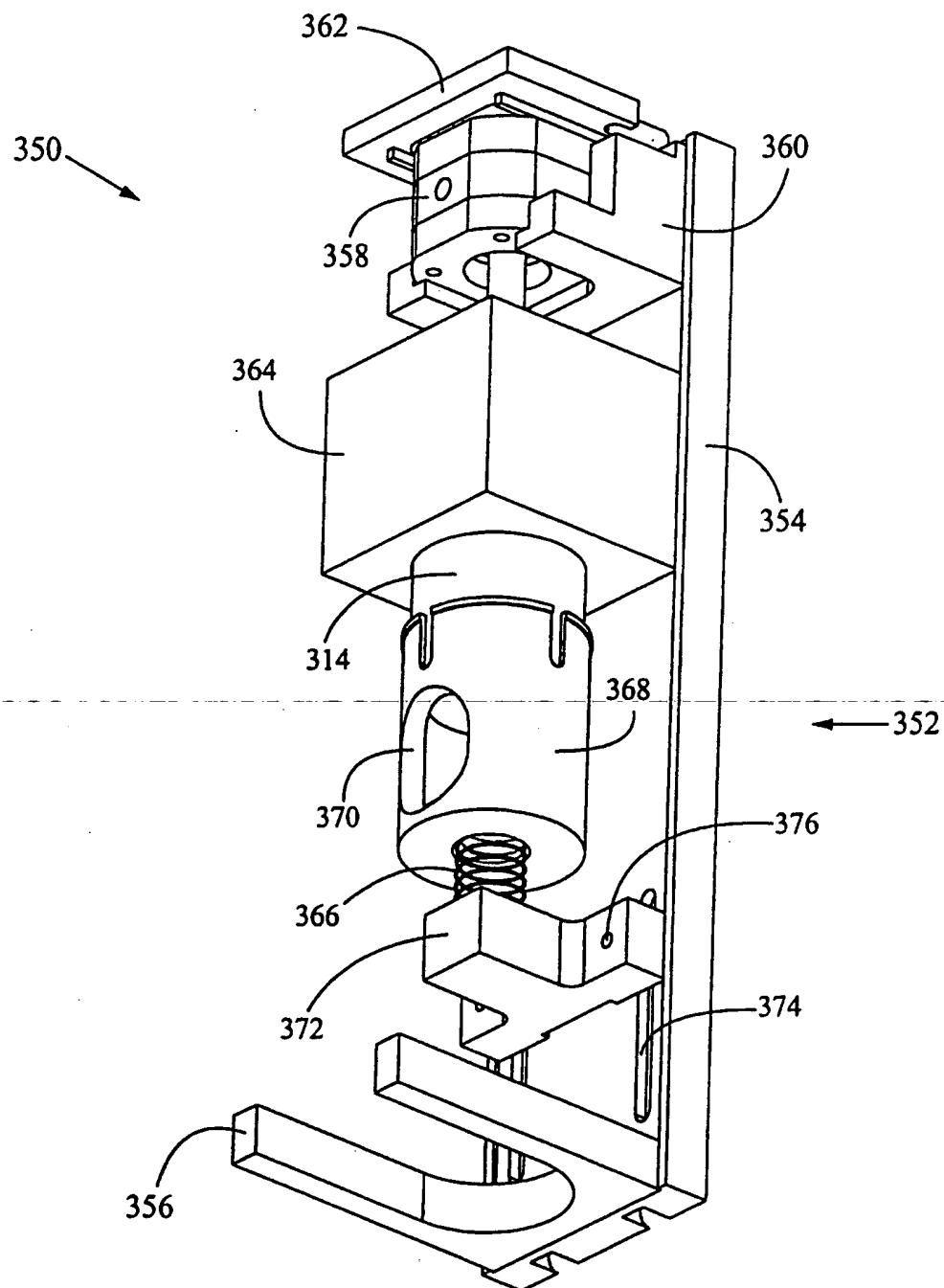


FIG. 36

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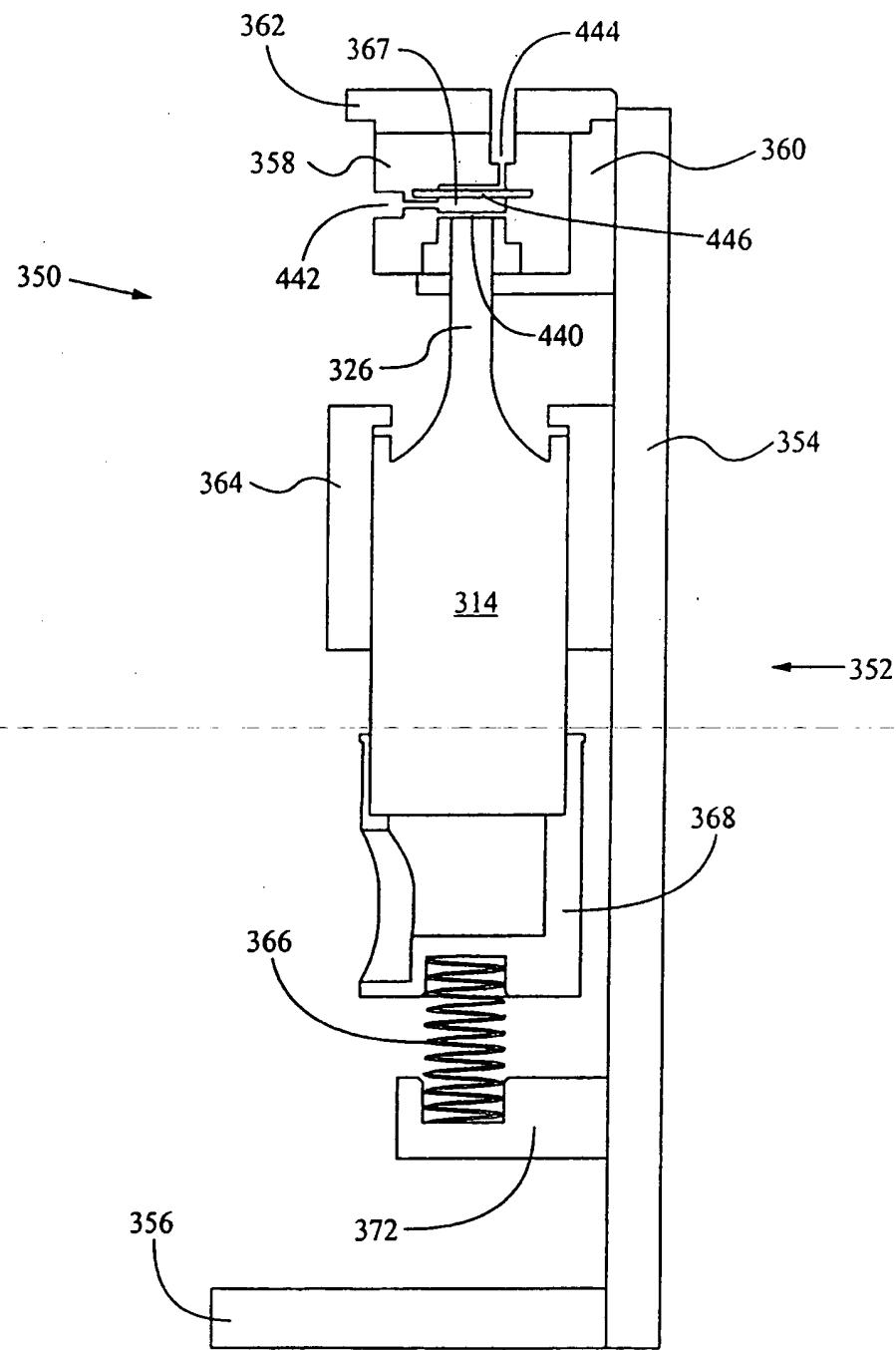


FIG. 37

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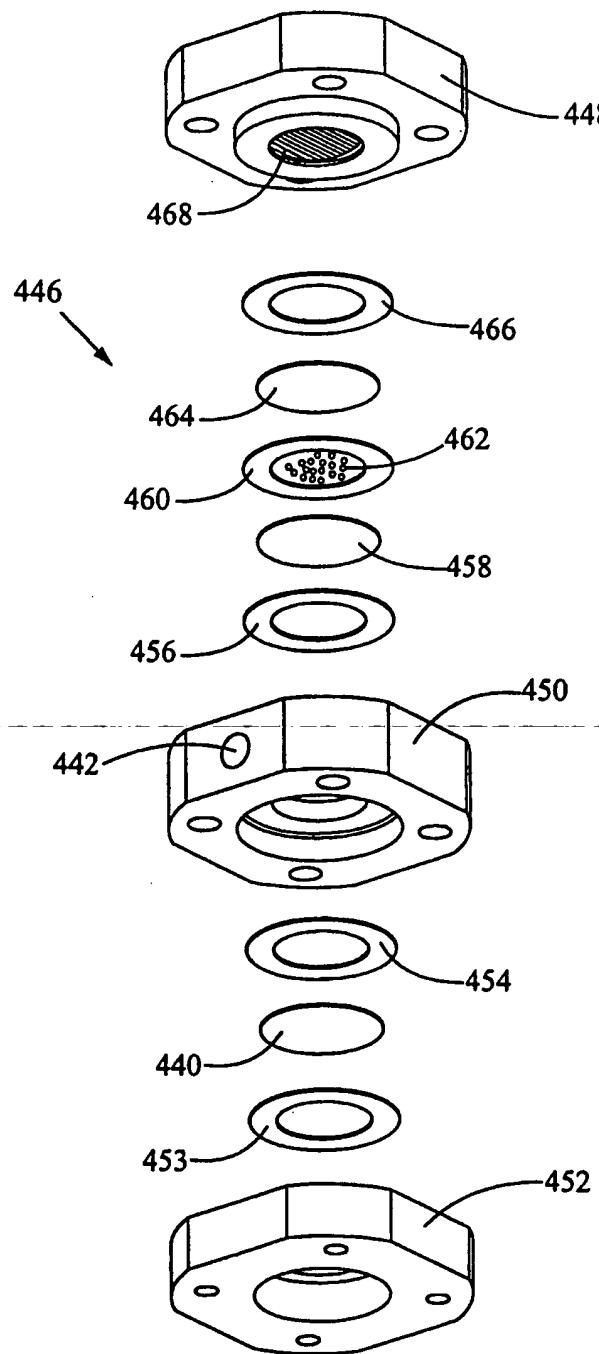


FIG. 38

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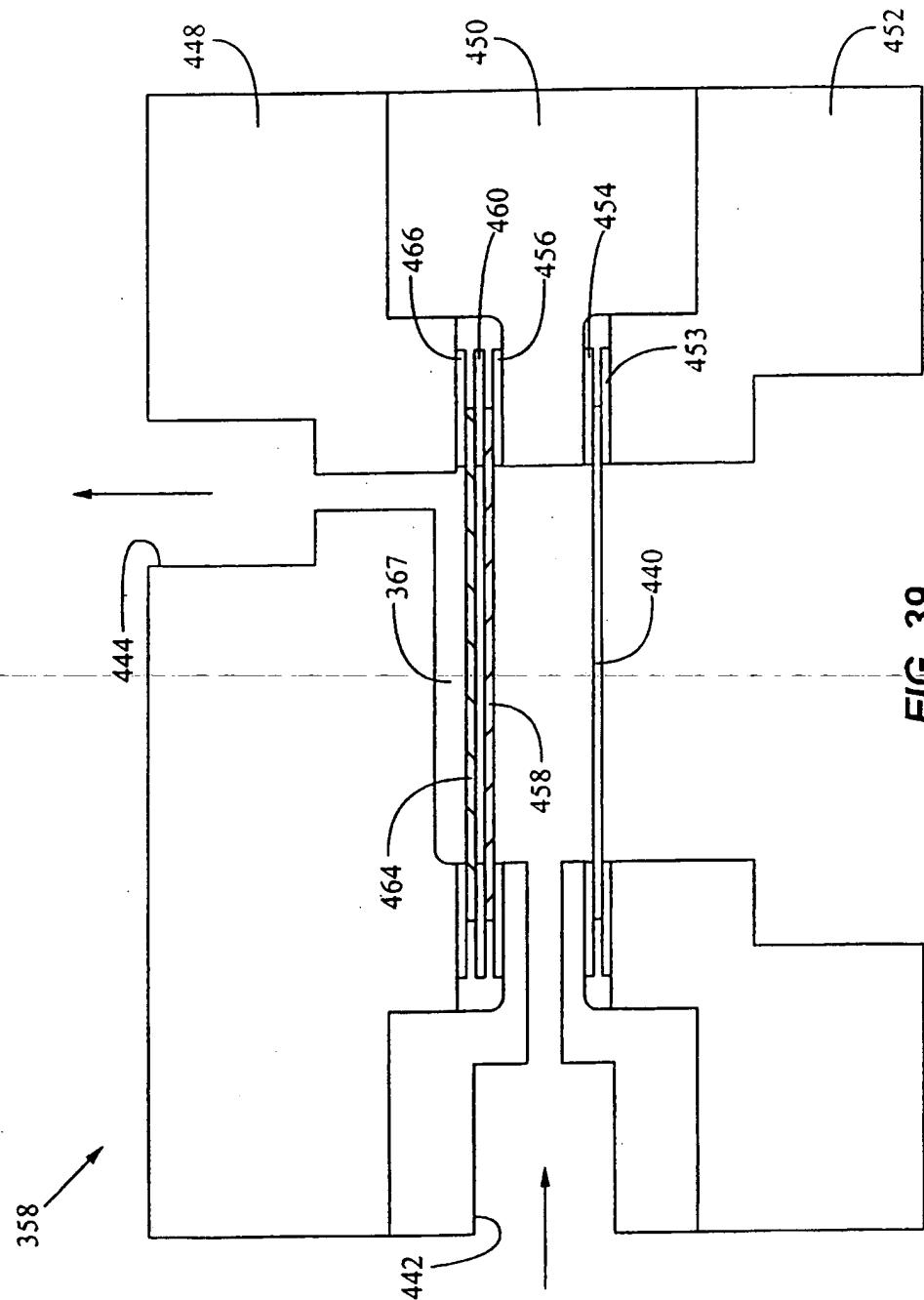


FIG. 39

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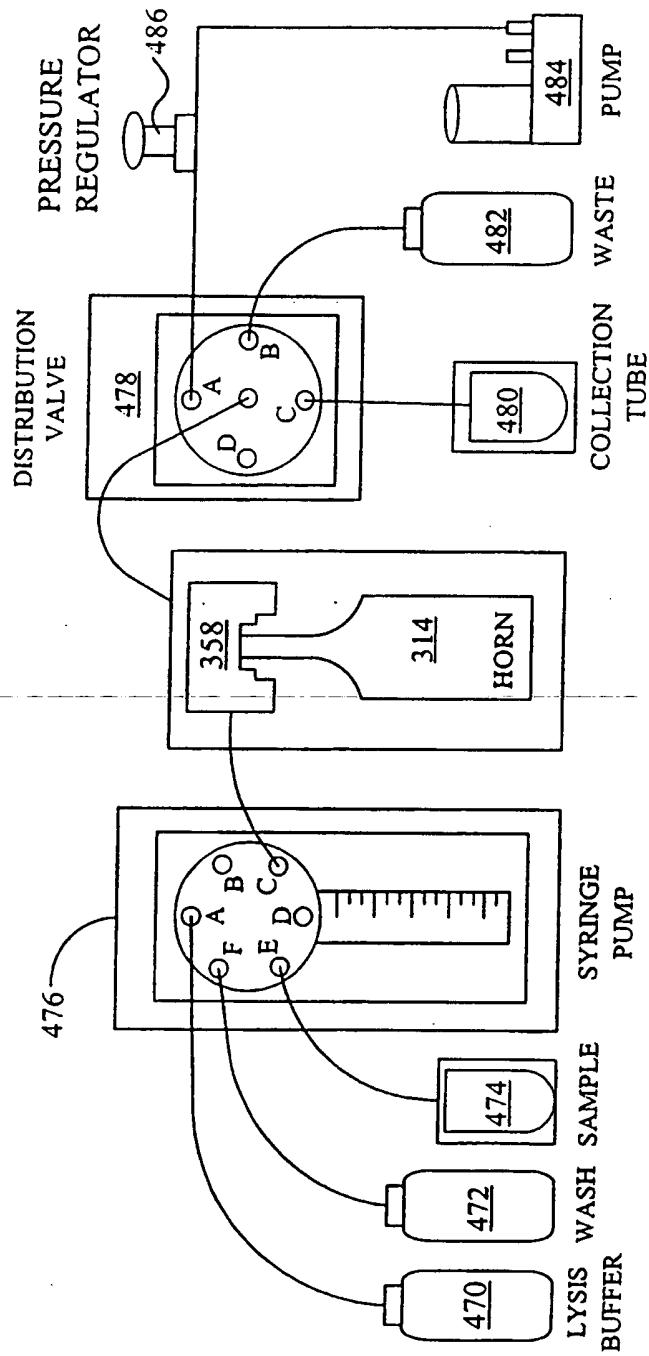


FIG. 40

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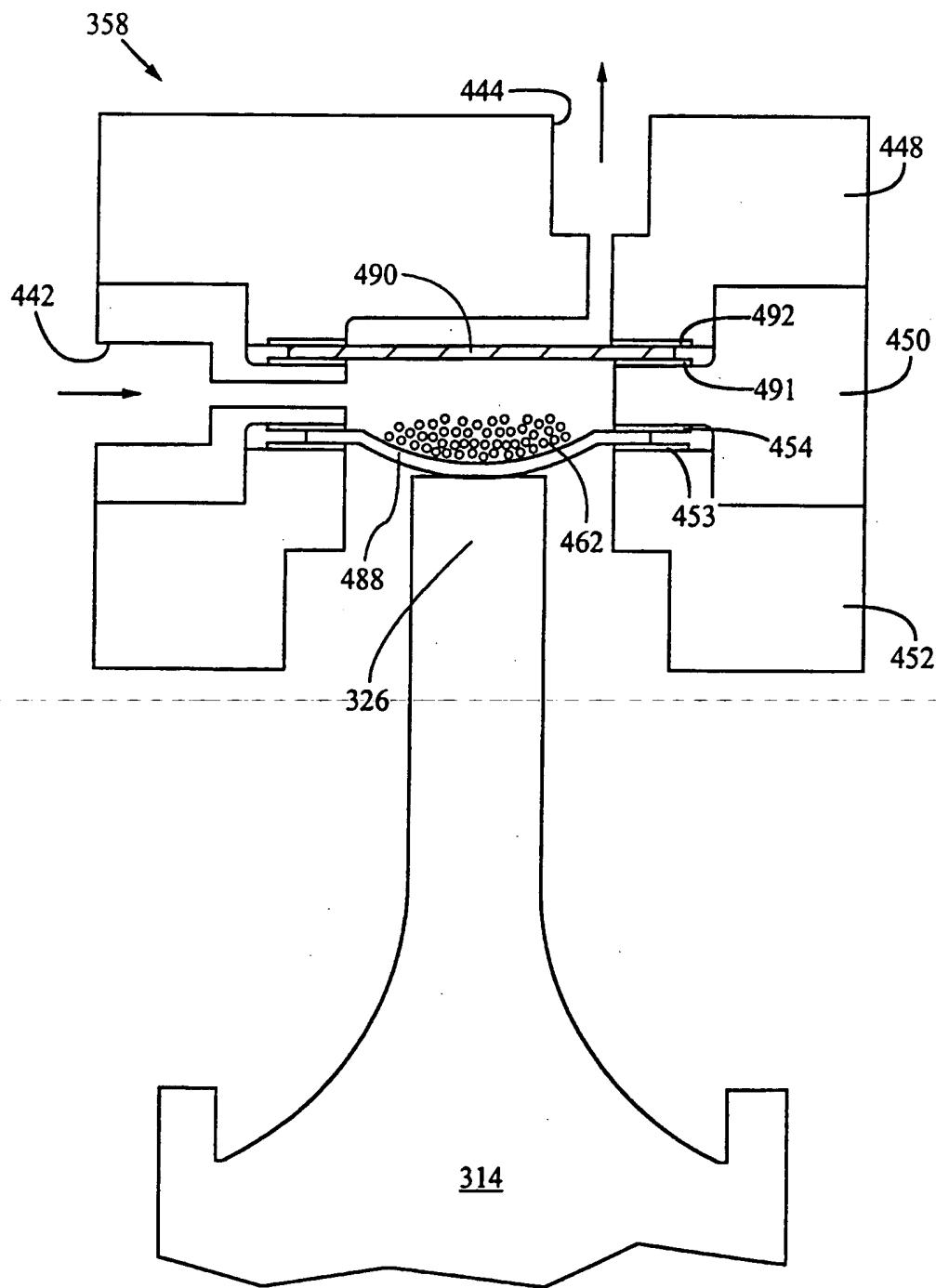


FIG. 41

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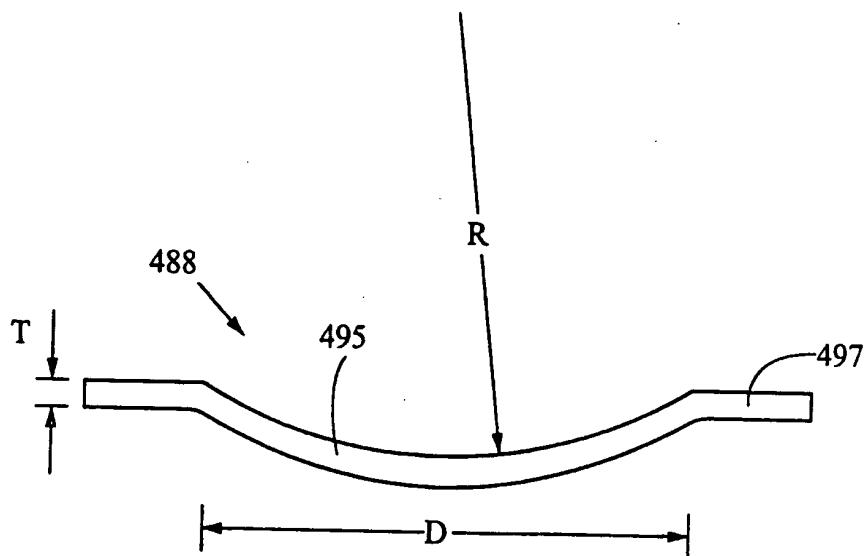


FIG. 42

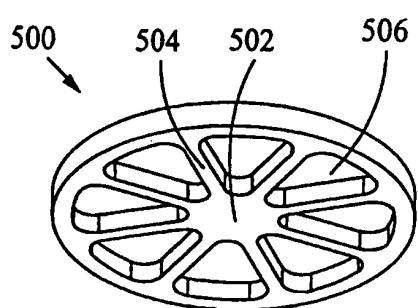


FIG. 43A

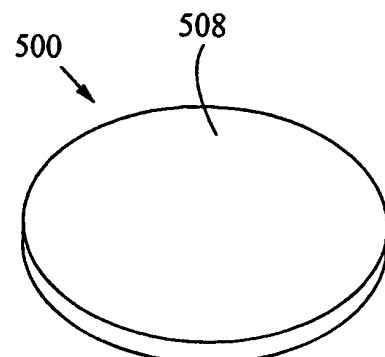


FIG. 43B

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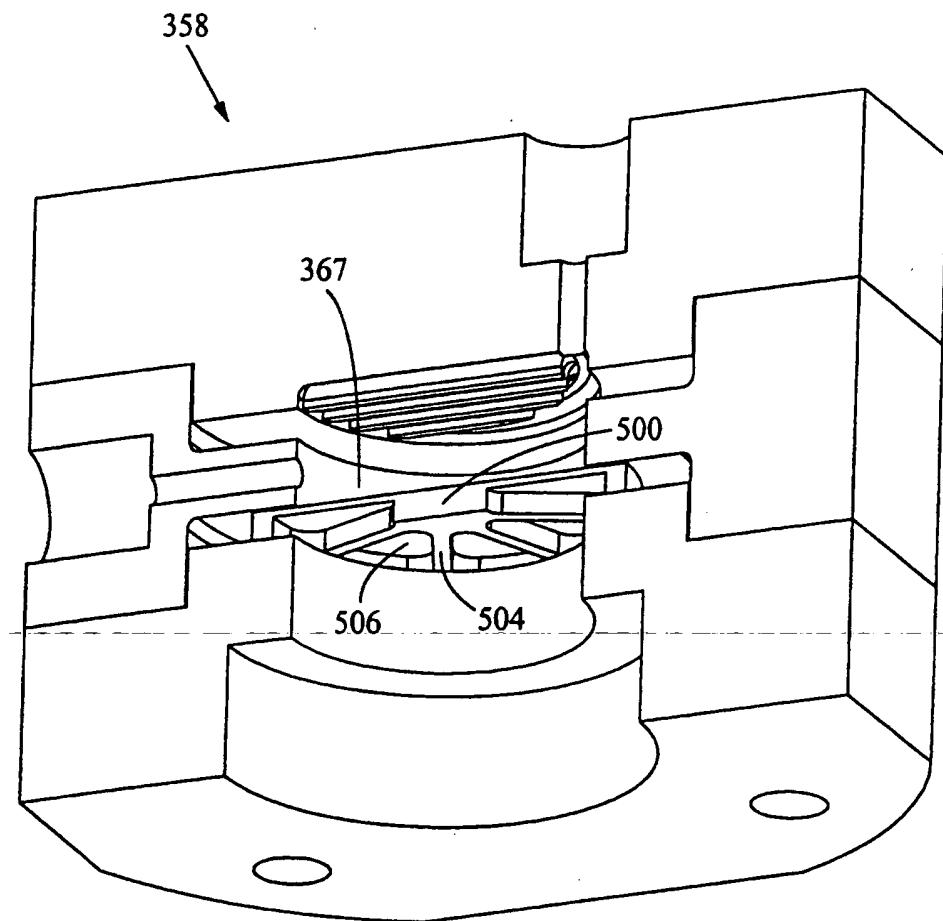
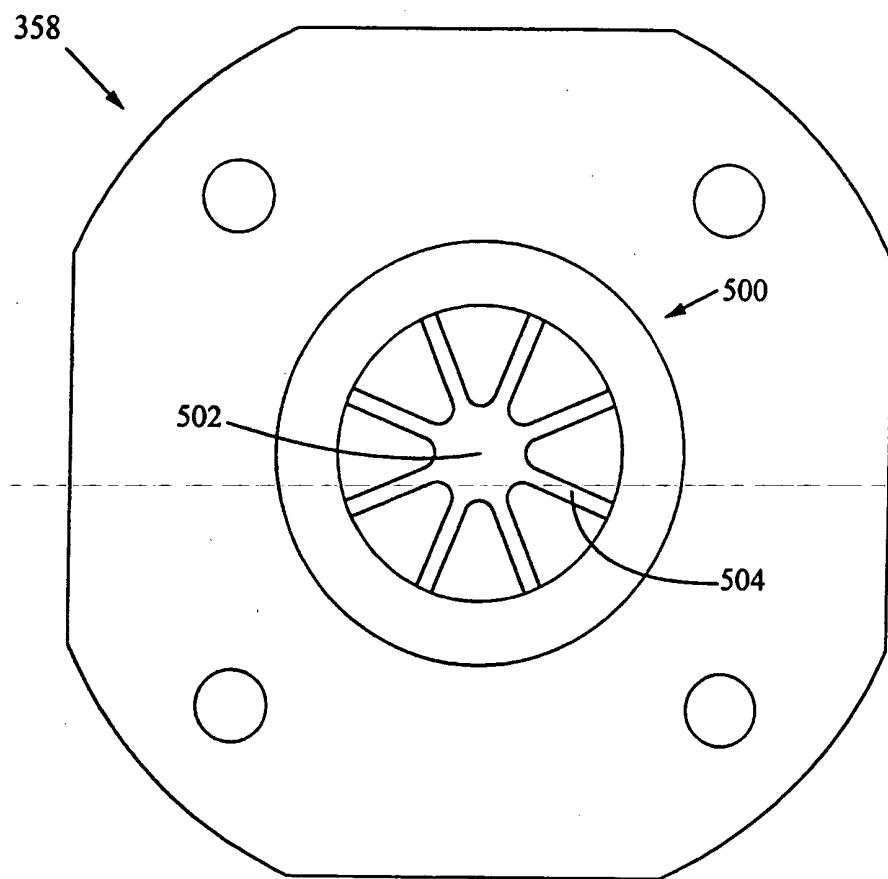


FIG. 44

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**FIG. 45**